(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 December 2002 (12.12.2002)

PCT

(10) International Publication Number
WO 02/099101 A1

(51) International Patent Classification[7]: C12N 15/00, 15/63, A61K 48/00

(74) Agents: FEILER, William, S. et al.; Morgan & Finnegan, L.L.P., 345 Park Avenue, New York, NY 10154 (US).

(21) International Application Number: PCT/US02/17258

(22) International Filing Date: 31 May 2002 (31.05.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/872,733 1 June 2001 (01.06.2001) US

(71) Applicant *(for all designated States except US)*: **THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES** [US/US]; Office of Technology Transfer, National Institutes of Health 6011 Executive Boulevard, Suite 325 Rockville, MD 20852 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **PAVLAKIS, George, N.** [US/US]; 9 Purdue Court, Rockville, MD 20850 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/099101 A1

(54) Title: MOLECULAR CLONES WITH MUTATED HIV GAG/POL, SIV GAG AND SIV ENV GENES

(57) Abstract: Nucleic acid constructs containing HIV-1 *gag/pol* and SIV *gag* or SIV *env* genes which have been mutated to remove or reduce inhibitory/instability sequences are disclosed. Viral particles and host cells containing these constructs and/or viral particles are also disclosed. The exemplified constructs and viral particles of the invention may be useful in gene therapy for numerous disorders, including HIV infection, or as a vaccine for HIV-1 immunotherapy and immunoprophylaxis.

2026-4287PC2

MOLECULAR CLONES WITH MUTATED HIV GAG/POL, SIV GAG AND SIV ENV GENES

I. TECHNICAL FIELD

The invention relates to nucleic acids comprising mutated HIV-1 *gag/pol* and SIV *gag* gene sequences which are capable of being expressed independently of any SIV or HIV regulatory factors. The invention also relates to nucleic acids comprising a mutated SIV *env* gene sequence, which is capable of being expressed independently of any SIV or HIV regulatory factors. The preferred nucleic acids of the invention are capable of producing infectious viral particles.

The invention also relates to vectors, vector systems and host cells comprising the mutated HIV-1 *gag*, HIV-1 *pol*, SIV *gag* and/or SIV *env* gene sequences. The invention also relates host cells comprising these nucleic acids and/or vectors or vector systems. The invention also relates to the use of these nucleic acids, vectors, vector systems and/or host cells for use in gene therapy or as vaccines.

II. BACKGROUND

Until recently, gene therapy protocols have often relied on vectors derived from retroviruses, such as murine leukemia virus (MLV). These vectors are useful because the genes they transduce are integrated into the genome of the target cells, a desirable feature for long-term expression. However, these retroviral vectors can only transduce dividing cells, which limits their use for *in vivo* gene transfer in nonproliferating cells, such as hepatocytes, myofibers, hematopoietic stem cells, and neurons.

Lentiviruses are a type of retrovirus that can infect both dividing and nondividing cells. They have proven extremely efficient at providing long-term gene expression (for up to 6 months) in a variety of nondividing cells (such as, neurons and macrophages) in animal models. See, e.g., Amado et al., Science 285:674-676 (July 1999). It has been proposed that the optimal gene transfer

system would include a vector based on HIV, or other lentivirus, that can integrate into the genome of nonproliferating cells. Because retroviruses integrate in the genome of the target cells, repeated transduction is unnecessary. Therefore, in contrast to an adenoviral vector capable of in vivo gene delivery, problems linked to the humoral response to injected viral antigens can be avoided. See, e.g., Naldini et al., Science, 272:263-267 (1996), p. 263.

HIV and other lentiviruses have a complex genome that, in addition to the essential structural genes (*env, gag,* and *pol*), contains regulatory (*tat* and *rev*) and accessory genes (*vpr, vif, vpu,* and *nef*). HIV has evolved to efficiently infect and express its genes in human cells, and is able to infect nondividing cells such as macrophages because its preintegration complex can traverse the intact membrane of the nucleus in the target cell. This complex contains, in addition to the viral DNA, the enzyme integrase, the product of the *vpr* gene, and a protein encoded by the *gag* gene called matrix. The matrix protein enables the preintegration complex to pass into the nucleus to access the host DNA. Lentiviruses cannot efficiently transduce truly quiescent cells (cells in the G_0 state). However, unlike murine retroviral vectors, in addition to being able to infect dividing cells, HIV-based vectors can achieve effective and sustained transduction and expression of therapeutic genes in nondividing cells, such as hematopoietic stem cells and in terminally differentiated cells such as neurons, retinal photoreceptors, muscle, and liver cells. See, e.g., Amado et al. (July 1999) and Klimatcheva et al., Frontiers in Bioscience 4:d481-496 (June 1999), and the references cited therein.

Although lentiviral vectors can be efficient gene delivery vehicles, there are safety concerns due to their origin. Therefore, the field has turned its attention to the development of vectors and production systems with built-in safety features to prevent the emergence of replication competent lentivirus (RCL). For example, in most laboratory applications, lentiviral vectors are generally created in a transient system in which a cell line is transfected with three separate constructs: a packaging construct, a transfer construct, and an envelope encoding construct. The packaging construct contains the elements necessary for vector packaging (except for env) and the enzymes required to generate vector particles. The transfer construct contains genetic cis-acting sequences necessary for the vector to infect the

target cell and for transfer of the therapeutic (or reporter) gene. The lentivirus *env* gene is generally deleted from the packaging construct and instead the envelope gene of a different virus is supplied in a third vector "the env-coding vector", although the lentiviruses *env* gene may be used if it is desired that the vector be intended to infect $CD4^+$T cells. A commonly used envelope gene is that encoding the G glycoprotein of the vesicular stomatitis virus (VSV-G), which can infect a wide variey of cells and in addition confers stability to the particle and permits the vector to be concentrated to high titers (see, e.g., Naldini et al., Science 272:263-267 (1996) and Akkina et al. J. Virol. 70:2581 (1996). The use of three separate constructs and the absence of overlapping sequences between them minimizes the possibility of recombination during lentivirus (transfer) vector production. In addition, because no viral proteins are expressed by the lentiviral (transfer) vector itself, they do not trigger an effective immune response against cells expressing vector in animal models (a particular problem with vectors based on adenovirus). See, e.g., Amado et al., Science 285:674-676 (July 1999) and the references cited therein. See also Naldini et al. Science 272:263-267 (1996).

The initial packaging plasmids contained most HIV genes except for *env*. In an effort to improve safety, subsequent HIV vectors have been produced in which the packaging plasmid is devoid of all accessory genes. This process does not interfere with efficient vector production and significantly increases the safety of the system because potential RCLs lack the accessory genes necessary for efficient replication of HIV in humans. Although these vectors can transduce growth-arrested cell lines and neurons *in vivo*, they have been reported to not efficiently transduce macrophages. The accessory gene *vpr* is believed to be necessary for HIV infection of these cells using these HIV vectors. See, Zufferey et al., Nature Biotechnol. 15:871-875 (1997). In contrast, as discussed later herein, the HIV-based lentiviral vectors of the present invention do not need any HIV accessory genes in order to be able to infect human macrophages and the other cells tested.

The requirement of *vpr* or *vif* for efficient transduction of liver cells has also been reported. See, e.g., Kafri et al., Nature Genet. 17:314 (1997). These results indicate that the requirement of accessory genes for efficient lentivirus-

mediated gene transfer is dependent on the type of cell chosen as target, suggesting that future applications of lentiviral vectors may involve vector constructs with different accessory genes, as needed.

Zufferey et al., (1997) describe an HIV vector system in which the virulence genes, *env, vif, vpr, vpu,* and *nef* have been deleted. This multiply attenuated vector conserved the ability to transduce growth-arrested cells and monocyte-derived macrophages in culture, and could efficiently deliver genes in vivo into adult neurons. The packaging plasmids described Zufferey et al. (1997) and Naldini et al. (1996) encode Rev and Tat, in addition to Gag and Pol.

Lentiviral vectors engineered to become packaged into virions in the absence of the regulatory gene *tat* have also been described. See, e.g., Kim et al., J. Virol. 72:811-816 (1998) and Miyoshi et al. J. Virol. 72:8150-8157 (1998). In these vectors the *tat* gene has been removed from the packaging plasmid. Kim et al. state that *tat* is not necessary as long as the serial 5' LTR promoter is replaced with a strong constitutive promoter. It also has other advantages for HIV therapy. Replacement of the HIV-1 LTR with a constitutive HCMV promoter permits the use of anti-Tat molecules such as Tat transdominant mutants or Tat activation response element decoys as therapeutic agents, since they will not affect vector production. (see p. 814, col. 2). The removal of the *tat* gene eliminates an essential virulence factor that could contribute to a possible RCL. Kim et al. (1998) describe a vector system which does not contain *tat, vif, vpr, vpu* and *nef.* The preferred vector system includes the *rev* gene which, the authors state "with RRE, is required for efficient RNA handling in this system." (p. 811, col. 2). However, Kim et al. also constructed Rev independent constructs using CTE. Kim et al. state that the *rev*/RRE components could be removed by using a sequence such as the Mason-Pfizer monkey virus (MPMV) constitutive transport element (CTE), thereby eliminating all accessory proteins, but this leads to a significant reduction in titer.

Srinivasakumar et al., J. Virol. 71:5841-5848 (1997) describes the generation of stable HIV-1 packaging lines that constitutively express high levels of HIV-1 structural proteins in either a Rev-dependent or a Rev-independent fashion. These cell lines were used to assess gene transfer by using a HIV-1 vector expressing the hygromycin B resistance gene and to study the effects of Rev, Tat,

and Nef on the vector titer. The Rev-independent cell lines were created by using *gag-pol* and *env* expression vectors that contain the MPMV CTE. This article describes the construction of four plasmids, among others: CMV gagpol-RRE and pCMVenv, which require Rev coexpression for HIV-1 structural gene expression, and pCMV gagpol-CTE and pCMVenv-CTE, which do not. To create Rev-containing and Rev-independent packaging, cell lines, CMT3 cells were transfected with vectors expressing Gag, Gag-Pol, and Env, using a calcium phosphate transfection procedure.

By creating an HIV vector which contained the MPMV CTE (pTR167-CTE) and a packaging cell line which expressed the HIV structural proteins in a Rev-independent fashion, the authors were able to obtain a HIV vector system that functions completely without Rev. The titer of the vector obtained from this system was essentially the same as that obtained from a parallel system which contained Rev. The authors state that, in this context, the CTE seemed to substitute completely for Rev-RRE functions, similar to what was previously observed in transient-expression assays with Rev-dependent constructs. This is in contrast to situations where several rounds of HIV replication were measured. In those cases, titers from CTE-containing viruses were always reduced by at least 1 log unit compared to viruses utilizing Rev and the RRE. (See, Srinivasakumar et al., p. 5847).

The authors state that the advantages of having a HIV vector system that works in the absence of Rev opens the possibility of using it as a delivery vehicle for intracellular immunization against Rev function. Genes encoding Rev antagonists that have dramatic inhibitory effects on HIV replication, such as Rev M10 or RRE decoys, could be introduced into an HIV vector and put into cells normally infectable by HIV. Expression of the "anti-Rev" gene would be expected to dampen HIV infection. Any residual HIV replication should lead to activation of the vector LTR (by Tat) and create a vector-derived RNA that would be packaged by proteins derived from the infectious virus. In this scenario, the wild-type virus would act as a helper that may allow the spread of vector particles to previously nonimmunized cells. Because of the additional vector spread, it is likely that this type of scheme will be more effective in modulating HIV infection *in vivo* than one

based on traditional retrovirus vectors. The authors state that they are currently testing this approach in model systems. (See, Srinivasakumar et al., p. 5847).

Another development in the quest for a safe system is the so-called self-inactivating (SIN) vector. See, e.g., Yu et al., Proc Natl Acad Sci USA 83:3194-8 (1986) and Miyoshi et al., J. Virol. 72:8150 (1998). In Yu et al., a retrovirus-derived vector SIN vector was designed for the transduction of whole genes into mammalian cells. The SIN vector of Yu et al. contains a deletion of 299 base pairs in the 3' long terminal repeat (LTR), which includes sequences encoding the enhancer and promoter functions. When viruses derived from such vectors were used to infect NIH 3T3 cells, the deletion was transferred to the 5' LTR, resulting in the transcriptional inactivation of the provirus in the infected cell. Introduction of a hybrid gene (human metallothionein-promoted c-fos) into cells via a SIN vector was not associated with rearrangements and led to the formation of an authentic mRNA transcript, which in some cases was induced by cadmium. The vector described in Miyoshi et al. also contains a deletion the 3' (downstream) LTR. A sequence within the upstream LTR serves as a promoter under which the viral genome is expressed. The deletion introduced in the downstream LTR is transferred to the upstream LTR during reverse transcription. This deletion inactivates the LTR promoter and eliminates the production of vector RNA. The gene (or genes) to be transferred (e.g., a reporter or therapeutic gene) is expressed from an exogenous viral or cellular promoter that is inserted into the lentivirus vector. An important safety feature of SIN vectors is that inactivation of the promoter activity of the LTR reduces the possibility of insertional mutagenesis (of the transfer vector) into the host genome. In addition, because the expression of the (transfer) vector RNA is eliminated, the potential for RCL production in the target cell is further minimized. SIN vectors should be particularly useful in gene transfer experiments designed to study the regulated expression of genes in mammalian cells. Absence of enhancer and promoter sequences in both LTRs of the integrated provirus should also minimize the possibility of activating cellular oncogenes and may provide a safer alternative to be used in human gene therapy. Other modifications to enhance safety and specificity include the use of specific internal promoters that regulate gene expression, either temporally or with tissue or cell specificity.

Other strategies to improve safety in human studies would be to use nonhuman lentiviruses such as simian immunodeficiency virus, bovine immunodeficiency virus, or equine infectious anemia virus. Of these, vectors derived from the feline immunodeficiency virus have been engineered to efficiently transduce nondividing human cells. See, e.g., Poeschla et al., Nature Med. 4:354-357 (1998) and WO 99/15641. In addition, White et al., J. Virol. 73:2832-2840 (April 1999) described lentiviral vectors using human and simian immunodeficient virus elements in attempt to improve safety by reducing the likelihood of recombination between packaging constructs and transfer constructs.

The development of efficient packaging lines has proven challenging because expression of the VSV-G envelope and a number of HIV proteins is toxic to cells. Recently, a producer line has been designed in which the expression of packaging genes and VSV-G, and therefore the production of vector, can be turned on at will. Kafri et al., J. Virol. 73-576-584 (1999). The cell line can be expanded for scale-up vector production when the expression of toxic genes is turned off. This cell line produces high titer vector without generating RCL. Hematopoietic stem cells transduced with an HIV vector were transplanted into rhesus macaques as described by Donahue et al. Blood 92 (suppl. 1), abstract 4648.5 (1998) with at least a 14-month follow-up. At that time the procedure proved to be safe; all animals in the study have remained healthy without evidence of circulating HIV or vector. See, Amado et al., Science 285:674-676 (July 1999).

Many gene therapy protocols have been designed to correct a number of inherited metabolic, infectious, or malignant diseases using the hematopoietic stem cell. This cell has the capacity to self-renew and to differentiate into all of the mature cells of the blood and immune systems. Many diseases that affect these systems could potentially be treated by the stable introduction of therapeutic genes into stem cells. Recently, lentiviral vectors were shown to bypass the need for *ex vivo* stem cell stimulation (which is necessary when using murine retroviral vectors), by mediating efficient gene transfer into very primitive human stem cells that contributed to stable, long-term reconstitution of SCID mouse bone marrow with many hematopoietic lineages. See, e.g., Miyoshi et al., Science 283:682 (1999). Similarly, in a rhesus macaque model of autologous transplantation with

lentivirus-transduced stem cells, multilineage gene expression was found, suggesting transduction of an early blood cell progenitor under conditions of minimal stem cell stimulation, ordinarily insufficient for transduction with murine retroviruses. See, Donahue et al., Blood 92 (suppl. 1), abstract 4648.5 (1999) and Amado et al., Science 285:674-676 (July 1999).

In HIV infection, another advantage of lentiviral vectors designed against HIV is their potential to be mobilized by HIV in the infected patient, because the virus supplies all of the necessary elements for packaging of the vector. If these mobilized vectors contained the HIV envelope, they could efficiently transfer their genes (for example, genes custom-designed to confer resistance against HIV) into $CD4^+$ T cells, protecting them from subsequent HIV infection. Lentiviral vectors can also be designed to efficiently express their genes only in $CD4^+$ T cells that are infected with HIV (so called *tat*-inducible vectors). In these vectors, all HIV genes, including *tat* and *rev*, are ablated; cis-acting sequences required for integration, expression, and packaging are retained, and expression is dependent on the activity of the HIV LTR (which requires transactivation by Tat). It has been shown that in this system, vector expression is induced efficiently upon HIV infection. Moreover, in the absence of genes that confer resistance against HIV, stable integration of this vector in permissive cell lines resulted in inhibition of HIV replication. Although the mechanism of HIV inhibition has not been completely elucidated, preliminary results suggest that this vector competes with HIV at the level of reverse transcription. See, An et al., J. Virol., in press, and Amado et al., Science 285:674-676 (1999).

A number of other potential medical applications, where the modification of the genetic material of quiescent cells could result in the prevention or reversal of a disease process, are beginning to be explored. For example, the finding that lentiviral vectors can mediate stable and long-term gene transfer by direct injection of vector into the rat and mouse retina has lent support to the notion of gene therapy for the treatment of retinitis pigmentosa. This degenerative disease of the retina is characterized by photoreceptor cell death, resulting in a slow progression to blindness. Mutations in the cGMP phosphodiesterase β subunit (PDEβ) gene of rod photoreceptors lead to an autosomal recessive form of retinitis

pigmentosa in humans, and in the *rd* mouse model of the disease. Previous studies have shown that adenovirus and adeno-associated virus-mediated PDEP subretinal gene transfer results in a delay in photoreceptor cell death. Using the *rd* mouse model, a recent study demonstrated that photoreceptors could be rescued in up to 50% of eyes injected with a lentivirus vector containing the murine PDEβ gene. In contrast with the short-term expression previously obtained with adenovirus vectors, PDEβ expression in this study persisted for at least 24 weeks. This finding points to the potential success of gene therapy in a disease that currently lacks effective treatment. See, Takahashi et al., J. Virol., 73:7812-7816 (Sept. 1999) and Amado et al. Science, 285:674-676 (1999).

In nature, the expression of *gag*, *pol*, and *env* of HIV-1 depends on the presence of the viral Rev protein. This dependence is, at least in part, due to the presence of negatively acting sequences (inhibitory or instability elements [INS]) located within unspliced and partially spliced mRNAs. The positive interaction of Rev with the Rev-responsive element [RRE] in these mRNAs counteracts the negative effects of the inhibitory sequences.

None of the above references teach or suggest that the *gag* and/or *pol* genes described therein may be replaced with the *gag* and/or *pol* genes in which the inhibitory/instability have been mutated to render their expression Rev-idependent. Furthermore, there is no disclosure of the specific HIV-1 *gag/pol* or SIV *gag* mutated genes described herein.

The *gag/pol* clone of the invention was made using the method for eliminating inhibitory/instability regions from a gene as first described in U.S. patent application Serial No. 07/858,747, filed March 27, 1992 (which issued as US Patent No. 6,174,666) entitled "Method of Eliminating Inhibitory/Instability Regions from mRNA" and later described in a Continuation-in-Part ("CIP") application, filed as PCT application PCT/US93/02908 on March 29, 1993 and U.S. Patent Nos. 5,972,596 and 5,965,726. The disclosure of the CIP application was published as International Publication No. WO 93/20212 on October 14, 1993. (The disclosures of these patents and patent applications are specifically incorporated by reference herein in their entirety.) The method was also described in Schwartz et al., J. Virol. 66:7176-7182 (1992).

Schneider et al., J. Virol. 71:4892-4903 (1997), extend the work described in the patent applications and in Schwartz et al. by identifying and characterizing additional INS within *gag*, *protease* and *pol* genes and mutating them in a similar manner. Schneider et al. disclose nucleic acid constructs which contain completely mutated HIV-1 *gag* genes, but only partially mutated HIV-1 *pol* genes.

Schneider et al. demonstrate that expression vectors containing an intact or nearly intact $p55^{gag}$ region allow the production of immature viral particles in mammalian cells in the absence of any other HIV proteins. The introduction of additional mutations in the *protease* region allowed efficient production of Gag/protease, which resulted in processing of the $Pr55^{gag}$ precursor and production of mature Gag particles with a lentivirus-like conical-core structure.

Schneider et al. disclose that Rev-independent expression vectors allow the efficient expression of Gag proteins in many cell lines that are not able to support efficient Rev-RRE-dependent rescue of these RNAs. Schneider et al. also disclose that *gag/pol* expression vectors may be important for vaccination approaches against HIV-1, since the *gag/pol* region is more conserved than is the *env* region and may be important for an effective immune response against HIV and for protection against infection. They also state that efficient HIV gene expression in many cells is also of interest for possible gene transfer experiments using lentiviral vectors in nondividing or slowly dividing cells, since HIV and the other lentiviruses are able to infect quiescent cells.

Pavlakis et al., Natl Conf Hum Retroviruses Relat Infect (2nd). (1995), 91, state that Rev-independent Gag expression vectors were able to produce viral particles in human and mouse cells in the absence of any other HIV proteins, and that additional mutations in the *pol* region allowed the expression of the protease and the processing of the p55 gag precursor. Direct DNA injection of TAT and Rev independent Gag expression vectors in mouse muscle resulted in Gag expression detected by ELISA and in anti-gag antibody response. Several Rev-and Tat- independent Gag expression cassettes were inserted into retroviral vectors and cell lines expressing Gag or Gag fragments that are dominant negative inhibitors of HIV-1 were constructed.

Shiver et al. (1996) describe the results of DNA vaccination of mice and non-human primates with mutated plasmid DNA encoding either mutated genes encoding HIV-1 gag (p55 gag) or env (gp120 or gp160). Both *gag* and *env* vaccine recipients exhibited antigen-specific cytotoxic and helper T lymphocyte (CTL, Th) responses. The results are stated to demonstrate that DNA vaccines elicited long-lived T cell responses in both mice and nonhuman primates that were disseminated throughout the lymphatics.

III. SUMMARY OF THE INVENTION

The invention relates to nucleic acids comprising the nucleic acid sequence of the mutated HIV-1 gag/pol gene shown in Figure 1 (SEQUENCE ID NO:1) and vectors and vector systems comprising these nucleic acids.

The invention also relates to nucleic acids comprising the nucleic acid sequence of the mutated SIV *gag* gene shown in Figure 3 and vectors and vector systems comprising these nucleic acids.

The invention also relates to nucleic acids comprising the mutated SIV *env* gene shown in Figure 17 and vectors and vector systems comprising these nucleic acids.

The invention also relates to products produced by the nucleic acids, e.g., mRNA, protein, and infectious viral particles.

The invention also relates to compositions comprising these nucleic acids and/or their expression products.

The invention also relates to host cells comprising these nucleic acids, vector systems or viral particles.

The invention also relates to uses of these nucleic acids, vector systems, host cells, expression products, and/or compositions to produce mRNA, proteins, and/or infectious viral particles, and/or to induce antibodies and/or cytotoxic or helper T lymphocytes.

The invention also relates to the use of these nucleic acid constructs, vectors, vector systems and or host cells for use in immunotherapy and immunoprophylaxis, e.g., as a vaccine, or in genetic therapy after expression, preferably in humans. The nucleic acid constructs of the invention can include or be

incorporated into lentiviral vectors or other expression vectors or they may also be directly injected into tissue cells resulting in efficient expression of the encoded protein or protein fragment. These constructs may also be used for *in-vivo* or *in-vitro* gene replacement, e.g., by homologous recombination with a target gene in-situ.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. DNA sequence of a mutated HIV-1 *gag/pol* molecular clone (SEQUENCE ID NO:1). The gagpol terminator is located at positions 4305-4307 of SEQUENCE ID NO:1.

Fig. 2. Comparison of the sequence of the wild –type and mutated *pol* region in pCMVgagpolBNkan. Position #1 in the figure is position 2641 in plasmid pCMVgagpolBNkan. The comparison starts at position 1872 from the gag initiator ATG.

Fig. 3. DNA sequence of a mutated SIV *gag* molecular clone (SIVgagDX).

Fig. 4. Comparison of the mutated SIV *gag* DNA sequence in SIVgagDX with the wild type SIV sequence from Simian (macaque) immunodeficiency virus isolate 239, clone lambda siv 239-1 (GenBank accession No. M33262).

Fig. 5. Schematic diagram of some components of sample versions of a lentiviral system. BGH poly (A): bovine growth hormone poly (A) signal; MSD: mutated splice donor site; ψ: encapsidation signal; SD, splice donor site; SA, splice acceptor site; "X" indicates that the ATG codon of the partial *gag* gene sequence is mutated so that translation of this gene does not occur.

Fig. 6. Schematic diagram of the packaging construct pCMVgagpolBNkan.

Fig. 7. Schematic diagram of transfer construct 1: pmBCwCNluci. The packaging signal, the CMV promoter and the coding region for the luciferase gene are flanked by the 5' and 3 HIV-1 LTRs, which provide promoter and polyadenylation signals, as indicated by the arrows. Three consecutive arrows indicate the U5, R, and U3 regions of the LTR, respectively. The transcribed

portions of the LTRs are shown in black. Some restriction endonuclease cleavage sites are also indicated.

Fig. 8. Schematic diagram of transfer construct 1: pmBCmCNluci. Symbols are as above.

Fig. 9. DNA sequence of packaging construct pCMVgagpolBNkan.

Fig. 10. DNA sequence of transfer construct 1: pmBCwCNluci.

Fig. 11. DNA sequence of transfer construct 1: pmBCmCNluci.

Figure 12:

Fig. 12. Nucleotide sequence of the region BssHII (711) to ClaI (830) in wild-type HIV-1 molecular clones HXB2 and NL4-3, and in the transfer constructs. The translation initiator signal for Gag protein (ATG) is underlined. pmBCwCNluci and pmBCmCNluci (transfer constructs 1 and 2) contain the sequence mBCwCN. Transfer construct 3 contains the sequence m2BCwCN. In contrast to the sequence mBCwCN, m2BCwCN has different mutations at the 5' splice site region and has an intact Gag ATG.

Fig. 13. Bar graph showing levels of gag protein that is released from cells upon transient transfection with pCMVgagpolBNkan (labeled pCMVBNKan in the figure).

Fig. 14. Bar graph showing reverse transcriptase activity from the Rev-independent gag-pol HIV-1 vector pCMVgagpolBNkan (labeled pCMVBNKan in the figure).

Fig. 15. Bar graphs showing the amount of luciferase per nanogram of p24 Gag protein detected in cells transducted with PCMVgagpolBNkan Rev-independent *gag*-HIV-1 based retroviral vectors. The results show that with PCMVgagpolBNkan Rev-independent gag-HIV-1 based retroviral vectors display high transduction efficiency in (A) 293 cells, (B) human lymphoid cells, (C) human myeloid cells (U937), as well as (D) non-dividing cells such as primary human macrophages.

Fig. 16. Schematic diagram of the SIV envelope encoding vector CMVkan/R-R-SIVgp160CTE.

Fig. 17. DNA sequence of the SIV envelope encoding vector CMVkan/R-R-SIVgp160CTE containing a mutated SIV env gene.

V. MODES FOR CARRYING OUT THE INVENTION

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only, and are not restrictive of the invention, as claimed. The accompanying drawings, which are incorporated in and constitute a part of the specification, illustrate an embodiment of the invention and, together with the description, serve to explain the principles of the invention.

One aspect of the invention comprises vectors that encode the Gag and/or Pol of HIV-1 in a Rev-independent manner. An example of such a vector which is described herein is the plasmid pCMVgagpolBNkan, which encodes the complete Gag and Pol of HIV-1 in a Rev-independent manner, and also contains a gene conferring kanamycin resistance. This plasmid is Tat and Rev-independent and was generated by eliminating the inhibitory/instability sequences present in the *gag/pol* mRNA without altering the amino acid sequence of the proteins coded by the genes.

The *gag/pol* clone of the invention is a DNA construct of the *gag/pol* region of HIV which has had the inhibitory/instability regions removed. The construct is expected to be useful as a component a new type of lentivirus vector for use in gene therapy or as a vaccine.

The *gag*, *pol* or *gag/pol* sequences of the invention can be highly expressed in human and other mammalian cells in the absence of any other regulatory and structural protein of HIV, including Rev. When the *gag/pol* sequences are combined with a sequence encoding an envelope protein, such as the VSV G protein or the HIV envelope protein (e.g., in the same vector or in another expression vector), infectious virus is produced after transfection into human cells. When a gene encoding a non-HIV envelope protein is used, for example, in the presence of the HIV *gag/pol* gene, the virus particles produced would contains only the HIV proteins Gag and Pol.

Lentiviral vectors or vector systems based on the *gag*, *pol* or *gag/pol* sequences of this invention, as exemplified by the Rev-independent pCMVgagpol BNkan construct described herein, may be used for gene therapy *in vivo* (e.g., parenteral inoculation of high titer vector) or *ex vivo* (e.g., *in vitro* transduction of

patient's cells followed by reinfusion into the patient of the transduced cells). These procedures are been already used in different approved gene therapy protocols.

The HIV *gag/pol* clone and SIV *gag* clone of the invention were made using the method for eliminating inhibitory/instability regions from a gene as described in U.S. Patent No. 6,174,666, and also in related U.S. Patent Nos. 5,972,596 and 5,965,726, which are incorporated by reference herein. This method does not require the identification of the exact location or knowledge of the mechanism of function of the INS. Generally, the mutations are such that the amino acid sequence encoded by the mRNA is unchanged, although conservative and non-conservative amino acid substitutions are also envisioned where the protein encoded by the mutated gene is substantially similar to the protein encoded by the non-mutated gene. The mutated genes can be synthetic (e.g., synthesized by chemical synthesis), semi-synthetic (e.g., a combination of genomic DNA, cDNA, or PCR amplified DNA and synthetic DNA), or recombinantly produced. The genes also may optionally not contain introns. The nucleic acids of the invention may also contain Rev-independent fragments of these genes which retain the desired function (e.g., for antigenicity of Gag or Pol, particle formation (Gag) or enzymatic activity (Pol)), or they may also contain Rev-independent variants which have been mutated so that the encoded protein loses a function that is unwanted in certain circumstances. In the latter case, for example, the gene may be modified to encode mutations (at the amino acid level) in the active site of reverse transcriptase or integrase proteins to prevent reverse transcription or integration. Rev-independent fragments of the *gag* gene are described in U.S. patent application Serial No. 07/858,747, filed March 27, 1992, and also in related U.S. Patent Nos. 5,972,596 and 5,965,726, which are incorporated by reference herein. If desired, the ATG initiation codon of any HIV accessory gene (e.g., *vif*), if present, may also be mutated.

In addition to being capable of producing HIV Gag and Pol proteins in the absence of Rev regulatory protein in a cell in vivo, the HIV *gag/pol* clone and SIV *gag* clone of the invention are also capable of producing HIV Gag and Pol proteins in the absence of any added cis acting transport element, such as CTE or CTE-like elements (collectively refered herein as RNA Transport Elements (RTE)).

Experiments indicate that the mutated vectors of the invention for SIV *gag* are far superior to those adding CTE (see Qiu et al., J Virol. 73:9145-52 (1999)).

The expression of the proteins encoded by these vectors after transfection into human cells may be monitored at both the level of RNA and protein production. RNA levels are quantitated by methods known in the art, e.g., Northern blots, S1 mapping or PCR methods. Protein levels may also be quantitated by methods known in the art, e.g., western blot or ELISA or fluorescent detection methods. A fast non-radioactive ELISA protocol can be used to detect gag protein (DUPONT or COULTER gag antigen capture assay).

At least three types of lentiviral vectors based on the *gag/pol* genes of the invention for use in gene therapy and/or as a vaccine are envisioned, i.e., lentiviral vectors having

a) no round of replication (i.e., a zero replication system)

b) one round of replication

c) a fully replicating system

For a system with no round of replication, a *gag/pol* gene, or separate *gag* and *pol* genes, or fragments of these genes, expressed using appropriate transcription units, e.g., a CMV promoter and a BGH poly (A) site. This will allow expression of the *gag/pol* unit (or *gag* or *pol* or fragment(s) thereof) for vaccine purposes. This expression can be accomplished without the production of any functional retroviral enzymes, provided that the appropriate mutation(s), e.g., a missense mutation, are introduced. In a zero replication system, a virus stock will be administered to the cells or animals of interest. For example, if one creates and uses a virus stock with the exemplified system using the packaging vector PCMVgagpolBNKan, the transfer construct pmBCwCNluci or pmBCmCNluci, and the envelope containing vector pHCMV-G, one obtains a zero replication system. The virus particles produced by such system can infect cells, and the reverse transcribed transfer construct DNA will go into the nucleus but, because the coding regions for viral structural proteins are not present, there will be no virus expression and replication (0 rounds). If one transfects cells *in vivo* with the same 3 DNAs, they will go to the nucleus, express viral proteins, make infectious virus particles and go out and infect another cell or cells (1 round). Since in vivo delivery of three

plasmids may result in lower expression, at least two different embodiments are envisioned. In the first, two plasmids may be used, e.g., MV1 shown in Fig. 5 and an envelope expression plasmid such as pHCMV-G. Other plasmids encoding functional envelopes from HIV, SIV, or other retroviruses can also be used. Transfection by the two plasmids results in infectious virus that can infect and integrate into new cells (1 round). The infected cells produce gagpol but virus propagation is not possible in the absence of env.

For a system with one round of replication, at least two additional embodiments are envisioned. In the first method, a combination of the genes, e.g., a *gag/pol* gene, an *env* encoding gene and, preferably, a gene encoding a reporter protein or other polynucleotide or protein of interest, are delivered into the cells of interest *in vivo*. As discussed above for the exemplified system, if one transfects cells *in vivo* with the same 3 DNAs, they will go to the nucleus, express viral proteins, make infectious virus particles, be released and infect another cell or cells (1 round).

In another embodiment, the same result (i.e., only one round of replication) can be obtained by using transfer vectors that have deletions in the 3' LTR and in which a heterologous-promoter (e.g., the CMV-promoter, or inducible promoter, or tissue-specific promoter), is used in place of the '3'LTR promoter. The mutations in the 3' LTR making it inactive upon reverse transcription and integration. This is because the integrated provirus derives both its 5' LTR and its 3' LTR from the 3' LTR of the starting (transfer) construct. (This is a well-known property of all retroviruses and has been used to make self-inactivating vectors (SIN)). There are several reasons one may want to inactivate the incoming LTR promoter, one of which is to use a different tissue specific or regulated promoter for expression of a gene of interest in the integrated provirus. Note that, with SIN vectors, if one uses a viral stock made *in vitro* after transfection into cells and collection of infectious virus, there will be no round of replication. If one transfects cells with the DNAs *in vivo*, there will be one round of replication. If functional *gag*, *pol*, or *env* are not included in the DNA mix, there will not be any infection at all (i.e., infectious viruses will not be made).

A fully replicating Rev-independent system has not been constructed yet, although it is expected that a functional system can be constructed using Rev-independent *gag/pol* and *env* sequences. If desired, extra posttranscriptional control elements such as the CTE element, which can replace Rev and give infectious virus (see e.g., Zolotukhin et al., J.Virol.68:944-7952 (1994)) are included. The fully replicating system should be in one piece, containing the LTR, packaging signal, gag/pol, splice site, env, tat, one or more CTE or CTE-like elements (if desired for optimal results), and LTR. Tat is thought to be required in this construct, at least in non-permissive cells. Such a system is depicted in Figure 5, (construct MV2). In this system, a cell or animal of interest (preferably human) would be infected with virus stock that then propagates. CTE or CTE-like elements (depicted in construct MV2 as RTE (RNA Transport Elements)) are desirable since they have been shown to improve expression, and since many retroviruses require the presence of posttranscriptional control elements. There are several types of CTE and CTE-like elements, and these elements appear to work via a different pathway from the Rev-RRE pathway. See, e.g., Tabernero et al., J Virol. 71:95-101 (1997). See also, Pavlakis and Nappi, PCT/US99/11082, filed May 22, 1999, published as WO 99/61596 on December 2, 1999 (and incorporated herein by reference), which describes a new type of post-transcriptional control element that is able to replace CTE and HIV RRE/Rev. The Pavlakis-Nappi element does not work in the same way as CTE and does not have any sequence or structure homology.

In a preferred embodiment, a lentiviral system of the invention comprises the following three components:

1. a packaging vector containing nucleic acid sequences encoding the elements necessary for vector packaging such as structural proteins (except for HIV *env*) and the enzymes required to generate vector particles, the packaging vector comprising at least a mutated HIV or SIV *gag/pol* gene of the invention;

2. a transfer vector containing genetic cis-acting sequences necessary for the vector to infect the target cell and for transfer of the therapeutic or reporter or other gene(s) of interest, the transfer vector comprising the encapsidation signal and the gene(s) of interest or a cloning site for inserting the gene(s) of interest;

and

3. a vector containing sequences encoding an element necessary for targeting the viral particle to the intended recipient cell, preferably the gene encoding the G glycoprotein of the vesicular stomatis virus (VSV-G) or amphotrophic MuLV or lentiviral *envs*.

Using the CMV promoter or other strong, high efficiency, promoter instead of the HIV-1 LTR promoter in the packaging vector, high expression of *gag, pol,* or *gag/pol* can be achieved in the total absence of any other viral protein. The exchange of the HIV-1 LTR promoter with other promoters is beneficial in the packaging vector or other vectors if constitutive expression is desirable and also for expression in other mammalian cells, such as mouse cells, in which the HIV-1 promoter is weak. Vectors containing the sequences of the invention can be used for the Rev independent production of HIV-1 Gag/Pol, HIV-1 Gag, HIV-1 Pol, and SIV Gag proteins. In certain embodiments, the presence of heterologous promoters will also be desired in the transfer vector and the envelope encoding vector, when such vectors are used.

The gene(s) of interest are chosen according to the effect sought to be achieved. For gene therapy purposes there will be at least one therapeutic gene encoding a gene product which is active against the condition it is desired to treat or prevent. Alternatively or additionally, there may be a gene which acts as a marker by encoding a detectable product. Therapeutic genes may encode, for example, an anti-sense RNA, a ribozyme, a transdominant negative mutant of a target protein, a toxin, a conditional toxin, an antigen that induces antibodies or helper T-cells or cytotoxic T-cells, a single chain antibody or a tumor suppresser protein. See, e.g., WO 98/17816.

An even more extensive list of genes of interest for use in lentiviral vectors is described, e.g., in WO 99/04026 on page 10, line 20 to page 12, line 7. Table 2 of Klimatcheva et al. (1999) also provides a list of disorders and target cells for gene therapy, as well as a number of lentiviral vectors used by others. This list includes genetic/metabolic deficiencies, viral infection and cancer. Inherited genetic defects such as adenosine deaminase deficiency, familial hypercholesterolemia, cystic fibrosis, mucopolysaccharidosis type VII, types I and II diabetes, classical phenylketonuria and Gaucher disease are diseases which are listed as being possible to overcome by lentiviral vector-mediated gene therapy because they constitute single-gene deficiencies for which the involved genes are known. Viral diseases are also listed as constituting appropriate targets for lentiviral gene delivery. In particular, a number of gene therapy approaches have been proposed for the treatment of HIV infection and, for some of these strategies, phase I studies have recently begun in humans. The article states that preliminary studies have dealt with defective murine oncoviruses for delivery of anti-sense RNAs, ribozymes and trans-dominant proteins against HIV replication.

In any of the vectors, but preferably in the transfer vector, an inserted gene could have an internal ribosomal entry site (IRES), e.g., from picornaviral RNA. An IRES will be used in circumstances that one wants to express two proteins from the same promoter. For example one protein of interest and a marker gene, e.g., green fluorescent protein (GFP) or a marker gene and a drug resistance gene (e.g. the firefly luciferase gene and neomycin phosphotransferase gene) as described on p. 58 of WO 99/04026, for example. Using an IRES the expression of the two proteins is coordinated. A further gene or genes may also be present under the control of a separate promoter. Such a gene may encode for example a selectable marker, or a further therapeutic agent which may be among the therapeutic agents listed above. Expression of this gene may be constitutive; in the case of a selectable marker this may be useful for selecting successfully transfected packaging cells, or packaging cells which are producing particularly high titers of the retroviral vector particles. Alternatively or additionally, the selectable marker may be useful for selecting cells which have been successfully infected with the lentiviral vector and have the provirus integrated into their own genome.

One way of performing gene therapy is to extract cells from a patient, infect the extracted cells with a lentiviral vector and reintroduce the cells back into the patient. A selectable marker may be used to provide a means for enriching for infected or transduced cells or positively selecting for only those cells which have been infected or transduced, before reintroducing the cells into the patient. This procedure may increase the chances of success of the therapy. Selectable markers may be for instance drug resistance genes, metabolic enzyme genes, or any other selectable markers known in the art. Typical selection genes encode proteins that confer resistance to antibiotics and other toxic substances, e.g., histidinol, puromycin, hygromycin, neomycin, methotrexate etc. and cell surface markers.

However, it will be evident that for many gene therapy applications of lentiviral vectors, selection for expression of a marker gene may not be possible or necessary. Indeed expression of a selection marker, while convenient for *in vitro* studies, could be deleterious *in vivo* because of the inappropriate induction of cytotoxic T lymphocytes (CTLs) directed against the foreign marker protein. Also, it is possible that for *in vivo* applications, vectors without any internal promoters will be preferable. The presence of internal promoters can affect for example the transduction titres obtainable from a packaging cell line and the stability of the integrated vector. Thus, single transcription unit vectors, which may be bi-cistronic or poly-cistronic, coding for one or two or more therapeutic genes, may be the preferred vector designed for use *in vivo*. See, e.g., WO 98/17816.

Suitable host or producer cells for use in the invention are well known in the art. May lentiviruses have already been split into replication defective genomes and packaging components. For those which have not the technology is available for doing so. The producer cell encodes the viral components not encoded by the vector genome such as the Gag, Pol and Env proteins. The *gag*, *pol* and *env* genes may be introduced into the producer cell transiently, or may be stably integrated into the cell genome to give a packaging cell line. The lentiviral vector genome is then introduced into the packaging cell line by transfection or transduction to create a stable cell line that has all of the DNA sequences required to produce a lentiviral vector particle. Another approach is to introduce the different

DNA sequences that are required to produce lentiviral vector particle, e.g., the *env* coding constrict, the *gag-pol* coding construct and the transfer construct into the cell simultaneously by transient triple transfection.

Target cells identified by Klimatcheva et al. (1999), and the references cited therein, include airway epithelial cells for cystic fibrosis; retinal photoreceptor cells for retinitis pigmentosa; progenitors for red blood cells, macrophages, and lymphocytes for hematopoietic disorders, sickle cell anemia, ß-thalassemia, lysosomal storage disorders, mucopolysaccharidoses, and severe combined immunodeficiency syndrome; bone marrow cells and macrophages for Gaucher's disease; liver cells for familial hypercholesterolaemia; T-lymphocytes and macrophages for HIV infection; brain tissue, neurons, and glial cells for neurodegenerative diseases such as Parkinson's and Alzheimer's diseases; endothelial cells and cardiac myocytes for cardiovascular diseases; and cancer cells in various tissues (e.g. liver or brain) for cancer. Target cells for other diseases would be apparent to one of skill in the art.

Vaccines and pharmaceutical compositions comprising at least one of the nucleic acid sequences, vectors, vector systems, or transduced or transfected host cells of the invention and a physiologically acceptable carrier are also part of the invention.

As used herein, the term "transduction" generally refers to the transfer of genetic material into the host via infection, e.g., in this case by the lentiviral vector. The term "transfection" generally refers to the transfer of isolated genetic material into cells via the use of specific transfection agents (e.g., calcium phosphate, DEAE Dextran, lipid formulations, gold particles, and other microparticles) that cross the cytoplasmic membrane and deliver some of the genetic material into the cell nucleus.

Systems similar to those described herein can be produced using elements of lentiviruses in addition to the HIV and/or SIV genes described herein.

Pharmaceutical Compositions

The pharmaceutical compositions of the invention contain a pharmaceutically and/or therapeutically effective amount of at least one nucleic acid

construct, vector, vector system, viral particle/virus stock, or host cell (i.e., agents) of the invention. In one embodiment of the invention, the effective amount of an agent of the invention per unit dose is an amount sufficient to cause the detectable expression of the gene of interest. In another embodiment of the invention, the effective amount of agent per unit dose is an amount sufficient to prevent, treat or protect against deleterious effects (including severity, duration, or extent of symptoms) of the condition being treated. The effective amount of agent per unit dose depends, among other things, on the species of mammal inoculated, the body weight of the mammal and the chosen inoculation regimen, as is well known in the art. The dosage of the therapeutic agents which will be most suitable for prophylaxis or treatment will also vary with the form of administration, the particular agent chosen and the physiological characteristics of the particular patient under treatment. The dose is administered at least once. Subsequent doses may be administered as indicated.

To monitor the response of individuals administered the compositions of the invention, mRNA or protein expression levels may be determined. In many instances it will be sufficient to assess the expression level in serum or plasma obtained from such an individual. Decisions as to whether to administer another dose or to change the amount of the composition administered to the individual may be at least partially based on the expression levels.

The term "unit dose" as it pertains to the inocula refers to physically discrete units suitable as unitary dosages for mammals, each unit containing a predetermined quantity of active material (e.g., nucleic acid, virus stock or host cell) calculated to produce the desired effect in association with the required diluent. The titers of the virus stocks to be administered to a cell or animal will depend on the application and on type of delivery (e.g., *in vivo* or *ex vivo*). The virus stocks can be concentrated using methods such as centrifugation. The titers to be administered *ex vivo* are preferably in the range of 0.001 to 1 infectious unit /cell. Another method of generating viral stocks is to cocultivate stable cell lines expressing the virus with the target cells. This method has been used to achieve better results when using traditional retroviral vectors because the cells can be infected over a longer period of time and they have the chance to be infected with multiple copies of the vector.

For *in vivo* administration of nucleic acid constructs, vectors, vector systems, virus stocks, or cells which have been transduced or transfected *ex vivo*, the dose is to be determined by dose escalation, with the upper dose being limited by the onset of unacceptable adverse effects. Preliminary starting doses may be extrapolated from experiments using lentiviral vectors in animal models, by methods known in the art, or may be extrapolated from comparisons with known retroviral (e.g., adenoviral) doses. Generally, small dosages will be used initially and, if necessary, will be increased by small increments until the optimum effect under the circumstances is reached. Exemplary dosages are within the range of 10^8 up to approximately 5 x 10^{15} particles.

Inocula are typically prepared as a solution in a physiologically acceptable carrier such as saline, phosphate-buffered saline and the like to form an aqueous pharmaceutical composition.

The agents of the invention are generally administered with a physiologically acceptable carrier or vehicle therefor. A physiologically acceptable carrier is one that does not cause an adverse physical reaction upon administration and one in which the nucleic acids are sufficiently soluble to retain their activity to deliver a pharmaceutically or therapeutically effective amount of the compound. The pharmaceutically or therapeutically effective amount and method of administration of an agent of the invention may vary based on the individual patient, the indication being treated and other criteria evident to one of ordinary skill in the art. A therapeutically effective amount of a nucleic acid of the invention is one sufficient to prevent, or attenuate the severity, extent or duration of the deleterious effects of the condition being treated without causing significant adverse side effects. The route(s) of administration useful in a particular application are apparent to one or ordinary skill in the art.

Routes of administration of the agents of the invention include, but are not limited to, parenteral, and direct injection into an affected site. Parenteral routes of administration include but are not limited to intravenous, intramuscular, intraperitoneal and subcutaneous. The route of administration of the agents of the invention is typically parenteral and is preferably into the bone marrow, into the CSF intramuscular, subcutaneous, intradermal, intraocular, intracranial, intranasal,

and the like. See, e.g., WO 99/04026 for examples of formulations and routes of administration.

The present invention includes compositions of the agents described above, suitable for parenteral administration including, but not limited to, pharmaceutically acceptable sterile isotonic solutions. Such solutions include, but are not limited to, saline and phosphate buffered saline for nasal, intravenous, intramuscular, intraperitoneal, subcutaneous or direct injection into a joint or other area.

In providing the agents of the present invention to a recipient mammal, preferably a primate, most preferably a human, the dosage administered will vary depending upon such factors as the mammal's age, weight, height, sex, general medical condition, previous medical history and the like.

The administration of the pharmaceutical compositions of the invention may be for either "prophylactic" or "therapeutic" purpose. When provided prophylactically, the compositions are provided in advance of any symptom. The prophylactic administration of the composition serves to prevent or ameliorate any subsequent deleterious effects (including severity, duration, or extent of symptoms) of the condition being treated. When provided therapeutically, the composition is provided at (or shortly after) the onset of a symptom of the condition being treated.

For all therapeutic, prophylactic and diagnostic uses, one or more of the agents of the invention, as well as antibodies and other necessary reagents and appropriate devices and accessories, may be provided in kit form so as to be readily available and easily used.

Where immunoassays are involved, such kits may contain a solid support, such as a membrane (e.g., nitrocellulose), a bead, sphere, test tube, rod, and so forth, to which a receptor such as an antibody specific for the target molecule will bind. Such kits can also include a second receptor, such as a labeled antibody. Such kits can be used for sandwich assays to detect toxins. Kits for competitive assays are also envisioned.

VI. INDUSTRIAL APPLICABILITY

Mutated genes of this invention can be expressed in the native host cell or organism or in a different cell or organism. The mutated genes can be introduced into a vector such as a plasmid, cosmid, phage, virus or mini-chromosome and inserted into a host cell or organism by methods well known in the art. In general, the mutated genes or constructs containing these mutated genes can be utilized in any cell, either eukaryotic or prokaryotic, including mammalian cells (e.g., human (e.g., HeLa), monkey (e.g., Cos), rabbit (e.g., rabbit reticulocytes), rat, hamster (e.g., CHO and baby hamster kidney cells) or mouse cells (e.g., L cells), plant cells, yeast cells, insect cells or bacterial cells (e.g., E. coli). The vectors which can be utilized to clone and/or express these mutated genes are the vectors which are capable of replicating and/or expressing the mutated genes in the host cell in which the mutated genes are desired to be replicated and/or expressed. See, e.g., F. Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley-Interscience (1992) and Sambrook et al. (1989) for examples of appropriate vectors for various types of host cells. The native promoters for such genes can be replaced with strong promoters compatible with the host into which the gene is inserted. These promoters may be inducible. The host cells containing these mutated genes can be used to express large amounts of the protein useful in enzyme preparations, pharmaceuticals, diagnostic reagents, vaccines and therapeutics.

Mutated genes or constructs containing the mutated genes may also be used for in-vivo or in-vitro gene therapy. For example, a mutated gene of the invention will produce an mRNA in situ to ultimately increase the amount of protein expressed. Such gene include viral genes and/or cellular genes. Such a mutated gene is expected to be useful, for example, in the development of a vaccine and/or genetic therapy.

The constructs and/or proteins made by using constructs encoding the mutated gag, env, and pol genes could be used, for example, in the production of diagnostic reagents, vaccines and therapies for AIDS and AIDS related diseases. The inhibitory/instability elements in the HIV-1 gag gene may be involved in the establishment of a state of low virus production in the host. HIV-1 and the other

lentiviruses cause chronic active infections that are not cleared by the immune system. It is possible that complete removal of the inhibitory/instability sequence elements from the lentiviral genome would result in constitutive expression. This could prevent the virus from establishing a latent infection and escaping immune system surveillance. The success in increasing expression of the entire *gag/pol* gene by eliminating the inhibitory sequence element suggests that one could produce lentiviruses without any negative elements. Such lentiviruses could provide a novel approach towards attenuated vaccines.

For example, vectors expressing high levels of Gag can be used in immunotherapy and immunoprophylaxis, after expression in humans. Such vectors include retroviral vectors and also include direct injection of DNA into muscle cells or other receptive cells, resulting in the efficient expression of gag, using the technology described, for example, in Wolff et al., Science 247:1465-1468 (1990), Wolff et al., Human Molecular Genetics 1(6):363-369 (1992) and Ulmer et al., Science 259:1745-1749 (1993). Further, the gag constructs could be used in transdominant inhibition of HIV expression after the introduction into humans. For this application, for example, appropriate vectors or DNA molecules expressing high levels of $p55^{gag}$ or $p37^{gag}$ would be modified to generate transdominant gag mutants, as described, for example, in Trono et al., Cell 59:113-120 (1989). The vectors would be introduced into humans, resulting in the inhibition of HIV production due to the combined mechanisms of gag transdominant inhibition and of immunostimulation by the produced gag protein. In addition, the gag constructs of the invention could be used in the generation of new retroviral vectors based on the expression of lentiviral gag proteins. Lentiviruses have unique characteristics that may allow the targeting and efficient infection of non-dividing cells. Similar applications are expected for vectors expressing high levels of env.

Identification of similar inhibitory/instability elements in SIV indicates that this virus is a convenient model to test these hypotheses. SIV similarly modified could be used in place of HIV in an effort to further minimize the possibility of rearrangement events that would lead to the generation of infectious HIV.

The following examples illustrate certain embodiments of the present invention, but should not be construed as limiting its scope in any way. Certain modifications and variations will be apparent to those skilled in the art from the teachings of the foregoing disclosure and the following examples, and these are intended to be encompassed by the spirit and scope of the invention.

EXAMPLE 1

Rev-Independent HIV-1 Gag/Pol Molecular Clone

Figure 1 shows the DNA sequence of a Rev-independent HIV-1 *gag/pol* molecular clone. This DNA sequence shown encodes the complete Gag and Pol of HIV-1 and can be expressed in a Rev-independent manner when operably linked to a promoter. The Rev-independent *gag* sequence was described in U.S. Patent Nos. 6,174,666, 5,972,596 and 5,965,726 and the Rev-independent *pol* sequence was generated by eliminating the inhibitory/instability sequences using the methods described in those patents. Others have reportedly made Rev independent *gag* sequences by optimizing codon usage for human cells (see, e.g., WO 98/34640).

Figure 2 shows an alignment of the sequence of the wild - type and mutated *pol* region in pCMVgagpolBNkan. Position #1 in the figure is position 2641 in plasmid pCMVgagpolBNkan.

The elimination of INS in *gag*, *pol* and *env* regions allows the expression of high levels of authentic HIV-1 structural proteins in the absence of the Rev regulatory factor of HIV-1.

EXAMPLE 2

Rev-Independent SIV Gag Molecular Clone

Figure 3 shows the DNA sequence of a Rev-independent SIV *gag* molecular clone, SIVgagDX. Figure 4 shows the comparison of wild type (WT) and mutant (SIVgagDX) sequences. The wild type SIV sequence is from Simian (macaque) immunodeficiency virus isolate 239, clone lambda siv 239-1 (GenBank accession No. M33262).

EXAMPLE 3

Rev-Independent SIV Env Molecular Clone

Figure 16 shows a schematic diagram, and figure 17 shows the DNA sequence, of the "env-coding" vector CMVkan/R-R-SIVgp160CTE, which is an example of a vector comprising a mutated lentiviral *env* gene sequence which is capable of being expressed independently of any SIV or HIV regulatory factors. "CMV" denotes the cytomegalovirus promoter; "SRV-CTE" denotes the constitutive transport element (CTE) of Simian Retrovirus Type 1; "all-STOP" denotes a sequence providing translational stops in all three reading frames; "BGH terminator" denotes the bovine growth hormone polyadenylation signal. Other posttranscriptional control elements can be used instead of the indicated SRV-CTE, for example the one described by Pavlakis and Nappi, PCT/US99/11082, filed May 22, 1999, which was published as WO 99/61596 on December 2, 1999 (and which is incorporated herein by reference).

As mentioned previously above, such a vector encoding a lentiviral *env* gene may be used if it is desired that the vector infect $CD4^+$T cells. Also as mentioned previously above, the CTE element (i.e., the SRV-CTE element in the case of vector CMVkan/R-R-SIVgp160CTE), can be replaced with another post-transcriptional control element, such as the Pavlakis-Nappi element, that is able to replace CTE and HIV RRE/Rev. See Pavlakis and Nappi, PCT/US99/11082, filed May 22, 1999, which was published as WO 99/61596 on December 2, 1999 (and which is incorporated herein by reference).

EXAMPLE 4

Lentivirial Vector System

Figure 5 is a schematic of some of the components of a preliminary version of the Rev-independent lentiviral vector system exemplified herein, including a packaging construct and three different transfer vectors which may be used. In the lentiviral system exemplified herein, the packaging construct also contains the gene for kanamycin resistance. The lentiviral system exemplified herein also contains the vector pHCMV-G, which is shown in Figure 5.

In the packaging construct shown in Figure 5, "CMV" denotes the cytomegalovirus promoter, "Gag" denotes the gag gene, which generates components of the virion core, "Pro" denotes "protease" "RT" denotes "reverse transcriptase," 'Int" denotes "integrase" and "BGH poly (A)" denotes the bovine growth hormone polyadenylation signal. The protease, reverse transcriptase, and integrase genes comprise the "pol" gene. In transfer construct 1, "LTR" denotes the HIV "long terminal repeat", which contains a HIV promoter; "mSD" denotes "mutated splice donor site," which is present in the construct so that splicing of the RNA transcript does not occur; "ψ" denotes the encapsidation signal; "wGA" denotes part of the wild-type *gag* gene which contains sequences believed to be necessary for encapsidation; "X" indicates that the ATG codon of the partial *gag* gene sequence is mutated so that translation of this gene does not occur; "CMV" denotes the cytomegalovirus promoter and luciferase is used as a reporter gene. Luciferase can be replaced with any gene of interest. Another HIV LTR is present at the 3' end of transfer construct 1. Replacement of this LTR in constructs such as the transfer construct 1, 2, or 3 with a promoter-enhancer deleted HIV LTR leads to inactivation of LTR after integration. Transfer construct 2 is similar to transfer construct 1, the difference being that a mutated part of the *gag* gene (denoted "mGa") is used instead of the wild-type part of the *gag* gene. Transfer construct 3 (pm2BCwCNluci) has different mutations at the 5' splice site and has an intact ATG codon so that translation of part of the mutated *gag* gene occurs. Transfer construct 3 also has a 5' CMV promoter instead of a 5' LTR promoter. This construct is expressed independent of the presence of HIV Tat protein. The transfer constructs expressed from the LTR promoter are partially dependent on Tat protein. In 293 cells significant expression can be achieved in the absence of Tat. See, e.g., Valentin et al., Proc. Natl Acad. Sci. U S A. 95:8886-91 (1988).

EXAMPLE 5

Generation of Packaging Construct pCMVgagpol BNkan

Figure 6 shows a schematic map of the packaging construct pCMV gagpolBNKan. The nucleotide numbering is that of the HXB2R sequence

(Genbank accession number K03455 and M38432), where +1 is the start of transcription.

The sequence in HIV-1 *gag/pol* region was mutated in order to eliminate all the INS. The fragment from the beginning of *gag* to BsrGI site in *pol*, and the fragment KE [KpnI(3700)- EcoRI(4194)] were previously mutated described in Schneider et al., J Virol. 71: 4892-4903 (1997) and in U.S. Patent Nos. 6,174,666, 5,972,596 and 5,965,726.

To generate pCMVgagpolBNkan, three fragments within HIV-1 *pol* region were mutated. They are fragment BP [BsrGI(2207)-PflMI(3032)], fragment PK [PflMI(3032)-KpnI(3700)] and fragment EN [EcoRI(4194)-NdeI(4668)]. Mutagenesis was performed using a modified version of the method described by Ho et al., Gene 77: 51-59 (1989) and DNA shuffling (Zhao and Arnold, Nucl. Acid Res. 25(6), 1307-1308 (1997). Sixteen oligonucleotides extending over the complete sequence of the three fragments were designed. Six oligos corresponded to fragment BP, six to fragment PK, and four to fragment EN (the oligonucleotides ranged from 130 to 195 bases in length; adjacent oligos overlapped by twenty nucleotides). Each fragment was assembled in two steps:

1) PCR; the reaction was carried out in standard *pfu* buffer with 10 pmol of each purified big oligo, 0.2 mM of each dNTPs and 2.5 u *pfu* DNA polymerase enzyme (Stratagene) in a 50 µl final volume. The PCR program was: 3 min 96°C followed by 50 cycles of 1 min 94°C, 1 min 55°C, and 1 min + 5 s/cycle 72°C, ended by 7 min at 72°C. After PCR, the big oligonucleotides were removed from the assembled mutated fragment.

2) The second step was to specifically amplify the assembled products with 30 mer primers located at the 5' and 3' end of each mutated fragment. One microliter of the assembled PCR product was used as template in a 25-cycle PCR reaction with 50 pmol of each primer, 1 x *pfu* buffer, 0.2 mM of each dNTP and 2.5 u *pfu* DNA polymerase in a 50 µl final volume. The PCR program was: 3 min 96°C, 10 cycles of 30 s 94°C, 30 s 55°C, 45 s 72°C, followed by another 14 cycles of 30 s 94°C, 30 s 55°C, 45 s + 20 s/cycle 72°C, and finally 7 min 72°C. This program gave a single PCR product of the correct size. The amplified BP, PK and EN fragments were individually cloned into PCR-script™ vector using PCR-

script™ Amp SK(+) Cloning Kit (Stratagene). Clones were randomly selected and sequenced. The correct BP, PK and EN fragments together with fragment KE previously mutated by Schneider et al. were ligated between BsrGI and KpnI site of p55AM1-R5 (which was previously described in Schneider et al., J. Virol. 71: 4892-4903 (1997)) to produce a completely mutated *gagpol* ORF. The new plasmid containing the completely mutated *gag/pol* was named pLTRgagpolBN. BN stands for the modification of the fragment between BsrGI and NdeI. The mutated *gag/pol* was then cloned into a CMVkan vector containing the cytomegalovirus major late promoter (GenBank accession no. X17403) and the kanamycin resistance gene, resulting in pCMVgagpolBNkan. The plasmid backbone comes from pVR1332 provided by Vical Inc., and described in Hartikka et al., Hum Gene Ther. 7:1205-17 (1996).

It is understood that different plasmid backbones can be used, e.g., to provide good expression *in vivo*, in the case of DNA injection, for example.

EXAMPLE 6

Construction of Transfer Vectors pmBCwCNluci and pmBCmCNluci

The HIV-1 sequence BC, between BssHII (257) and ClaI (376), contains the major splice donor site and the encapsidation signal. Six oligos (33 to 46 bases) were designed to introduce mutations on the splice donor site and the AUG start codon of *gag*. The BC fragment was assembled, amplified and sequenced as described in the section concerning the construction of pCMVgagpolBN.

The mutated BC fragment and a fragment of wild type *gag* between ClaI (376) and Nsi (793) were placed between the BssHII and Nsi sites of p55RRE (Schneider et al., J. Virol. 71:4892-4903 (1997)) to generate pmBCwCN. In parallel, the fragment between ClaI (376) and NsiI sites of mutated *gag* from p55BM1-10SD+ was used to generate pmBCmCN. (p55BM1-10SD+ is similar to p55BM1-10, which is described in Schneider et al. (1997), but contains in addition the intact splice donor and encapsidation site upstream of gag). The region between NsiI and XhoI containing 3' part of *gag* and RRE in pmBCwCN and pmBCmCN

was replaced by a ClaI-XhoI fragment containing CMV promoter and luciferase gene from pHR'-CMVluci (vector from D. Trono) to generate pmBCwCNluci and pmBCmCNluci (which are shown as transfer constructs 1 and 2 in Figure 5, and schematically depicted in Figures 7 and 8, respectively). The sequences of these plasmids are shown in Figures 10 and 11, respectively. Different versions of these plasmids have also been created, by standard procedures, with variations in the region of the encapsidation site, the first splice donor site, and the initiator *gag* AUG. For example, the transfer construct pm2BcwCNluci (which is shown as transfer construct 3 in Fig. 5) has different mutations in the 5` splice site region and has an intact ATG. A comparison of the sequences in the BssHII-Cla I region of transfer constructs 1 and 2 (mBCwCN frag), transfer construct 3 (m2BCwCN frag), HXB2 and NL43 is shown in Fig. 12.

EXAMPLE 7

Preparation of Viral Particles

Lentiviral particles were generated by transient cotransfection of 293 human kidney cells with a combination of three plasmids: pCMVgagpolBNkan, pmBCwCNluci or pmBCmCNluci (transfer vector) and pHCMV-G (Yee et al., Proc. Natl. Acad. Sci., USA, 91:9564-9568 (1994) a plasmid coding for the envelope VSV-G (glycoprotein of vesicular stomatitis virus).

The day before the transfection, 293 cells were plated at a density of 10^6 cells/plate on a 60 mm plate. Plasmid DNA was transfected by the Ca-phosphate precipitation method in the following proportions: 3 μg packaging construct, 6 μg transfer construct and 100 ng VSV-G encoding construct, pHCMV-G. [Note that the LTR promoter can be expressed in 293 cells in the absence of Tat with a moderate decrease in efficiency. The transfer constructs can be fully Tat independent after replacement of the LTR promoter with a CMV (see, e.g., transfer construct 3 in Fig. 5) or other promoter in such a way that the mRNA start site is at the beginning of the LTR R region.] In the present experiments for preparation of viral particles 500 ng of a Tat expression plasmid was included in the transfection.

Cells were washed the day after transfection and were kept in DMEM medium for another 48 hours before the supernatants were harvested. Supernatants were spun at 1,200 rpm for 7 mins to eliminate any floating cells. pCMVgagpolBNkan produces high levels of Gag protein that is efficiently released from the cells (Figure 13), and also produces high levels of functional Pol as judged by levels of reverse transcriptase activity similar to those found upon expression of complete HIV-1 (Figure 14).

Supernatants from 293 transfected cells were used to transduce several human cell lines (293, Jurkat, U937) and non-dividing human primary macrophages.

EXAMPLE 8

Cell Transduction

Transduction was performed by incubating for 3-4 hours at 37°C the target cells with 1-2 ml of supernatant containing the retroviral vectors. The amount of retroviral vector present in the supernatant was normalized by p24 content (measured by ELISA). Equal amounts of p24 gag protein were used for infection of cells. This way, differences in production of the different preparations was minimized.

The macrophages used for transduction were isolated from the peripheral blood of healthy donors by adherence to plastic. Cells were cultured in RPMI + 20% fetal calf serum (FCS) + 10% human serum (HS). After 1 week, non-adherent cells were washed off with PBS and the macrophages were kept in culture for another 1-2 weeks in the absence of human serum. The cells were washed 2-4 times with PBS before transduction.

Cells were harvested 48 hours after transduction (seven days for primary macrophages) and the transduction efficiency was determined by measuring luciferase activity in cell extracts from the cultures. The results of the transduction experiments in 293 Jurkat, U937 and primary macrophages are shown in Figure 15A-D. These results demonstrate that Rev-independent *gag*-HIV-1 based retroviral vectors display high transduction efficiency in (A) 293 cells, (B) human

lymphoid cells, (C) human myeloid cells (U937), as well as (D) non-dividing cells such as primary human macrophages.

EXAMPLE 9

Use Of Nucleic Acids of the Invention In Immunoprophylaxis Or Immunotherapy

In postnatal gene therapy, new genetic information has been introduced into tissues by indirect means such as removing target cells from the body, infecting them with viral vectors carrying the new genetic information, and then reimplanting them into the body; or by direct means such as encapsulating formulations of DNA in liposomes; entrapping DNA in proteoliposomes containing viral envelope receptor proteins; calcium phosphate co-precipitating DNA; and coupling DNA to a polylysine-glycoprotein carrier complex. In addition, in vivo infectivity of cloned viral DNA sequences after direct intrahepatic injection with or without formation of calcium phosphate coprecipitates has also been described. mRNA sequences containing elements that enhance stability have also been shown to be efficiently translated in Xenopus laevis embryos, with the use of cationic lipid vesicles. See, e.g., J.A. Wolff, et al., Science 247:1465-1468 (1990) and references cited therein.

Recently, it has also been shown that injection of pure RNA or DNA directly into skeletal muscle results in significant expression of genes within the muscle cells. J.A. Wolff, et al., Science 247:1465-1468 (1990). Forcing RNA or DNA introduced into muscle cells by other means such as by particle-acceleration (N. -S. Yang, et al. Proc. Natl. Acad. Sci. USA 87:9568-9572 (1990); S.R. Williams et al., Proc. Natl. Acad. Sci. USA 88:2726-2730 (1991)) or by viral transduction should also allow the DNA or RNA to be stably maintained and expressed. In the experiments reported in Wolff et al., RNA or DNA vectors were used to express reporter genes in mouse skeletal muscle cells, specifically cells of the quadriceps muscles. Protein expression was readily detected and no special delivery system was required for these effects. Polynucleotide expression was also obtained when the composition and volume of the injection fluid and the method of injection were modified from the described protocol. For example, reporter enzyme activity was

reported to have been observed with 10 to 100 μl of hypotonic, isotonic, and hypertonic sucrose solutions, Opti-MEM, or sucrose solutions containing 2mM $CaCl_2$ and also to have been observed when the 10- to 100- μl injections were performed over 20 min. with a pump instead of within 1 min.

Enzymatic activity from the protein encoded by the reporter gene was also detected in abdominal muscle injected with the RNA or DNA vectors, indicating that other muscles can take up and express polynucleotides. Low amounts of reporter enzyme were also detected in other tissues (liver, spleen, skin, lung, brain and blood) injected with the RNA and DNA vectors. Intramuscularly injected plasmid DNA has also been demonstrated to be stably expressed in non-human primate muscle. S. Jiao et al., Hum. Gene Therapy 3:21-33 (1992).

It has been proposed that the direct transfer of genes into human muscle in situ may have several potential clinical applications. Muscle is potentially a suitable tissue for the heterologous expression of a transgene that would modify disease states in which muscle is not primarily involved, in addition to those in which it is. For example, muscle tissue could be used for the heterologous expression of proteins that can immunize, be secreted in the blood, or clear a circulating toxic metabolite. The use of RNA and a tissue that can be repetitively accessed might be useful for a reversible type of gene transfer, administered much like conventional pharmaceutical treatments. See J.A. Wolff, et al., Science 247:1465-1468 (1990) and S. Jiao et al., Hum. Gene Therapy 3:21-33 (1992).

It had been proposed by J.A. Wolff et al., supra, that the intracellular expression of genes encoding antigens might provide alternative approaches to vaccine development. This hypothesis has been supported by a recent report that plasmid DNA encoding influenza A nucleoprotein injected into the quadriceps of BALB/c mice resulted in the generation of influenza A nucleoprotein-specific cytotoxic T lymphocytes (CTLs) and protection from a subsequent challenge with a heterologous strain of influenza A virus, as measured by decreased viral lung titers, inhibition of mass loss, and increased survival. J. B. Ulmer et al., Science 259:1745-1749 (1993).

Therefore, it appears that the direct injection of RNA or DNA vectors encoding the viral antigen can be used for endogenous expression of the antigen to generate the viral antigen for presentation to the immune system without the need for self-replicating agents or adjuvants, resulting in the generation of antigen-specific CTLs and protection from a subsequent challenge with a homologous or heterologous strain of virus.

CTLs in both mice and humans are capable of recognizing epitopes derived from conserved internal viral proteins and are thought to be important in the immune response against viruses. By recognition of epitopes from conserved viral proteins, CTLs may provide cross-strain protection. CTLs specific for conserved viral antigens can respond to different strains of virus, in contrast to antibodies, which are generally strain-specific.

Thus, direct injection of RNA or DNA encoding the viral antigen has the advantage of being without some of the limitations of direct peptide delivery or viral vectors. See J.A. Ulmer et al., supra, and the discussions and references therein). Furthermore, the generation of high-titer antibodies to expressed proteins after injection of DNA indicates that this may be a facile and effective means of making antibody-based vaccines targeted towards conserved or non-conserved antigens, either separately or in combination with CTL vaccines targeted towards conserved antigens. These may also be used with traditional peptide vaccines, for the generation of combination vaccines. Furthermore, because protein expression is maintained after DNA injection, the persistence of B and T cell memory may be enhanced, thereby engendering long-lived humoral and cell-mediated immunity. For example, Shriver et al. (1996) describes long-lived T cell responses in mice and non-human primates treated with vaccine compositions containing Rev-independent HIV *gag* and HIV *gag* vectors and Hel et al., *J. Immunol.*, 167:7180-7181 (2001), specifically incorporated by reference herein, describes Rev-independent expression SIV *gag* and SIV *env* vectors and their potentiation of SIV-specific $CD4^+$ and $CD8^+$ T cell responses in both naïve and infected macaques by a DNA-SIV and NYVAC-SIV prime/boost regimen.

1. Vectors for the immunoprophylaxis or immunotherapy against HIV-1

The mutated *gag*, *pol* or *gag/pol* sequences will be inserted in expression vectors using a strong constitutive promoter such as CMV or RSV, or an inducible promoter such as HIV-1.

The vector will be introduced into animals or humans in a pharmaceutically acceptable carrier using one of several techniques such as injection of DNA directly into human tissues; electroporation or transfection of the DNA into primary human cells in culture (ex vivo), selection of cells for desired properties and reintroduction of such cells into the body, (said selection can be for the successful homologous recombination of the incoming DNA to an appropriate preselected genomic region); generation of infectious particles containing the *gag* gene, infection of cells *ex vivo* and reintroduction of such cells into the body; or direct infection by said particles in vivo.

Substantial levels of protein will be produced leading to an efficient stimulation of the immune system.

In another embodiment of the invention, the described constructs will be modified to express mutated Gag proteins that are unable to participate in virus particle formation. It is expected that such Gag proteins will stimulate the immune system to the same extent as the wild-type Gag protein, but be unable to contribute to increased HIV-1 production. This modification should result in safer vectors for immunotherapy and immunophrophylaxis.

EXAMPLE 10

Inhibition of HIV-1 Expression Using Transdominant (TD)-TD-Gag-TD Rev or Td Gag-Pro-TD Rev Genes

Direct injection of DNA or use of vectors other than retroviral vectors will allow the constitutive high level of trans-dominant Gag (TDgag) in cells. In addition, the approach taken by B.K. Felber et al., Science 239:184-187 (1988) will allow the generation of retroviral vectors, e.g. mouse-derived retroviral vectors, encoding HIV-1 TDgag, which will not interfere with the infection of human cells by the retroviral vectors. In the approach of Felber, et al., supra, it was

shown that fragments of the HIV-1 LTR containing the promoter and part of the polyA signal can be incorporated without detrimental effects within mouse retroviral vectors and remain transcriptionally silent. The presence of Tat protein stimulated transcription from the HIV-1 LTR and resulted in the high level expression of genes linked to the HIV-1 LTR.

The generation of hybrid TDgag-TDRev or TDgag-pro-TDRev genes and the introduction of expression vectors in human cells will allow the efficient production of two proteins that will inhibit HIV-1 expression. The incorporation of two TD proteins in the same vector is expected to amplify the effects of each one on viral replication. The use of the HIV-1 promoter in a matter similar to one described in B.K. Felber, et al., supra, will allow high level Gag and Rev expression in infected cells. In the absence of infection, expression will be substantially lower. Alternatively, the use of other strong promoters will allow the constitutive expression of such proteins. This approach could be highly beneficial, because of the production of a highly immunogenic gag, which is not able to participate in the production of infectious virus, but which, in fact, antagonizes such production. This can be used as an efficient immuniprophylactic or immunotherapeutic approach against AIDS.

Examples of trans-dominant mutants are described in Trono et al., Cell 59:112-120 (1989).

1. Generation of constructs encoding transdominant Gag mutant proteins

Gag mutant proteins that can act as trans-dominant mutants, as described, for example, in Trono et al., supra, will be generated by modifying vector p37M1-10D or p55M1-13P0 to produce transdominant Gag proteins at high constitutive levels.

The transdominant Gag protein will stimulate the immune system and will inhibit the production of infectious virus, but will not contribute to the production of infectious virus.

The added safety of this approach makes it more acceptable for human application.

VII. REFERENCES

U.S. Patent No. 6,174,666 issued January 16, 2001 (Pavlakis and Felber)

U.S. Patent No. 5,972,596 issued October 26, 1999 (Pavlakis and Felber)

U.S. Patent No. 5,965,726 issued October 12, 1999 (Pavlakis and Felber)

WO 98/17816 Lentiviral Vectors (Kingsman & Kingsman) (Oxford Biomedica Ltd)

WO 98/34640 (Shiver, J.W., Davies, M-E M., Freed, D.C., Liu, M.A. and Perry,H.C. - Merck & Co., Inc.)

WO 98/46083 Use of Lentiviral Vectors for Antigen Presentation in Dendritic Cells (Wong-Staal, Li; Kan-Mitchell) (Univ. of Cal.)

WO 99/04026 Lentiviral Vectors (Chen, Gasmi, Yee and Jolly) (Chiron)

WO 99/15641 Non-Primate Lentiviral Vectors and Packaging Systems (Poeschia, Looney and Wong-Staal) (Univ. of Cal.)

WO 99/30742 Therapeutic Use of Lentiviral Vectors (Naldini and Song)

WO 99/51754 Infectious Pseudotyped Lentiviral Vectors Lacking Matrix Protein and Uses Thereof (Goettlinger, Reil and Bukovsky) (Dana Farber Cancer Inst Inc)

PCT/US99/11082 Post-Transcriptional Regulatory Elements and Uses Thereof (Pavlakis and Nappi), filed May 22, 1999, published as WO 99/61596 on December 2, 1999

Akkina, R.K., Walton, R.W., Chen, M.L., Li, Q-X, Planelles, V and Chen, I.S.Y., "High-efficiency gene transfer into $CD34^+$ cells with a human immunodeficiency virus type 1-based retroviral vector pseudotyped with vesicular stomatitis virus envelope glycoprotein G," *J. Virol.* 70:2581-2585 (1996)

Amado, R.G. & Chen, I.S.Y., "Letinviral vectors—the promise of gene therapy within reach?," *Science* 285:674-676 (July 1999)

Donahue, R.E., An, D.S., Wersto, R.P., Agricola, B.A., Metzger, M.E. and Chen, I.S.Y., "Transplantation of immunoselected $CD34^+$ cells transduced with a EGFP-expressing lentiviral vector in non-human primates," *Blood* 92(suppl. 1):383b, Abstract #4648.5 (1998)

Fox, J.L., "Researchers wary of fear-based ban on lentivirus gene therapy," *Nature Biotechnology* 16:407-408 (1998)

Goldman, M.J., Lee, P.S., Yang, J.S. & Wilson, J.M., "Lentiviral vectors for gene therapy of cystic fibrosis," *Hum Gene Ther.* 8, 2261-2268 (1997)

Hartikka J., Sawdey M., Cornefert-Jensen F., Margalith M., Barnhart K., Nolasco M., Vahlsing H.L., Meek J., Marquet M., Hobart P., Norman J., and Manthorpe M., "An improved plasmid DNA expression vector for direct injection into skeletal muscle," *Hum Gene Ther.* 7:1205-17 (1996)

Hel Z., Tsai W.P., Thornton A., Nacsa J., Giuliani L., Tryniszewska E., Poudyal M., Venzon D., Wang X., Altman J., Watkins D.I., Lu W., von Gegerfelt A., Felber B.K., Tartaglia J., Pavlakis G.N., and Franchini G.,

"Potentiation of simian immunodeficiency virus (SIV)-specific CD4(+) and CD8(+) T cell responses by a DNA-SIV and NYVAC-SIV prime/boost regimen," *J. Immunol.* 167(12):7180-91 (2001)

Kafri, T., Blomer, U., Peterson, D.A., Gage, F.H. & Verma, I.M., "Sustained expression of genes delivered directly into liver and muscle by lentiviral vectors," *Nat Genet.* 17, 314-317 (1997)

Kafri, T., van Praag, H., Ouyang, L., Gage, F.G. and Verma, I.M., "A packaging cell line for lentivirus vectors," *J. Virol.* 73:576-584 (1999)

Kim, V.N., Mitrophanous, K., Kingsman, S.M., and Kingsman, A.J., "Minimal Requirement for a Lentivirus Vector Based on Human Immunodeficiency Virus Type 1", *J. Virol.* 72:811-816 (1998)

Klimatcheva, E., Rosenblatt, JD. and Planelles, V., "Lentiviral vectors and gene therapy," *Frontiers in Bioscience* 4:d481-496 (June 1999)

Miyoshi, H., Takahashi, M., Gage, F.H. & Verma, I.M., "Stable and efficient gene transfer into the retina using an HIV-based lentiviral vector," *Proc Natl Acad Sci USA.* 94: 10319-10323 (1997)

Miyoshi, H., Blomer,U., Takahashi, M., Gage, F.H., and Verma, I.M., "Development of self-inactivating lentivirus vector," ," *J Virol.* 72:8150-8157 (1998)

Miyoshi, H., Smith, K.A., Mosier, D.E., Verma, I.M. and Torbett, B.E., "Transduction of human $CD34^+$ cells that mediate long-term engraftment of NOD/SCID mice by HIV vectors," *Science* 283:682-686 (1999)

Naldini, L., Blomer, U., Gallay, P., Ory, D., Mulligan, R., Gage, F.H., Verma, I.M. & Trono, D., "In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector," *Science*. 272, 263-267 (1996)

Naviaux, R.K, Costanzi, E., Haas, M. and Verma, I., "The pCL vector system: rapid production of helper-free, high-titer, recombinant retroviruses," *J. Virol.* 70:5701-5705 (1996)

Pavlakis, G.N., Schneider, R.; Song, S., Nasioulas, G., Zolotukhin, A., Felber, B.K., Trauger, R., Cox, J., and Manthorpe, M., "Use of simple Rev-independent HIV-1 gag expression vectors in gene therapy and gene vaccine applications," *Natl Conf Hum Retroviruses Relat Infect (2nd),* Jan 29-Feb 2 (1995); 91.

Poeschla, E.M., Wong-Staal, F. & Looney, D.J., "Efficient transduction of nondividing human cells by feline immunodeficiency virus lentiviral vectors," *Nature Med.* 4:354-357 (1998)

Qiu, J. T., R. Song, M. Dettenhofer, C. Tian, T. August, B. K. Felber, G. N. Pavlakis and X. F. Yu, "Evaluation of novel human immunodeficiency virus type 1 Gag DNA vaccines for protein expression in mammalian cells and induction of immune responses," *J Virol.* 73: 9145-52 (Nov. 1999)

Reynolds, P.N. and Curiel, D.T., "Viral vectors show promise in Colorado," *Nature Biotechnology* 16:422-423 (1998)

Schneider, R., Campbell, M., Nasioulas, G., Felber, B.K., and Pavlakis, G.N., Inactivation of the human immunodeficiency virus type 1 inhibitory elements allows Rev-independent expression of Gag and Gag/protease and particle formation, "*J. Virol.* 71:4892-4903 (1997)

Schwartz, S., M. Campbell, G. Nasioulas, J. Harrison, B. K. Felber and G. N. Pavlakis, "Mutational inactivation of an inhibitory sequence in human immunodeficiency virus type-1 results in Rev-independent *gag* expression," *J. Virol.* 66:7176-7182 (1992)

Shiver, J.W., Yasutomi, Y., Free, D.C., Davies, M.-E., Perry, H.C., Pavlakis, G.N., Letvin, N.L., and Liu, M.A., "DNA Vaccine-Mediated Cellular Immunity Against HIV-1 *gag* and *env*", presented at the Conference on Advances in AIDS Vaccine Development: 8th Annual Meeting of the National Cooperative Vaccine Development Groups for AIDS (NCVDGs) from February 11-15, 1996.

Soneoka, Y., Cannon, P.M., Ransdale, E.E., Griffiths, J.C., Romano, G., Kingsman, S.M. and Kingsman, A.J., "A transient three-plasmid expression system for the production of high titer retroviral vectors," *Nuc. Acids Res.* 23:628-633 (1995).

Srinivasakumar, N., Chazal, N., Helga-Maria, C., Prasad, S., Hammarskjöld, M.-L., and Rekosh, D., "The Effect of Viral Regulatory Protein Expression on Gene Delivery by Human Immunodeficiency Virus Type 1 Vectors Produced in Stable Packaging Cell Lines," *J. Virol.*, 71:5841-5848 (1997)

Sutton, R.E., Wu, H.T., Rigg, R., Bohnlein, E. & Brown, P.O., "Human immunodeficiency virus type 1 vectors efficiently transduce human hematopoietic stem cells," *J. Virol.* 72, 5781-5788 (1998)

Tabernero, C., A. S. Zolotukhin, J. Bear, R. Schneider, G. Karsenty and B. K. Felber, "Identification of an RNA sequence within an intracisternal-A particle element able to replace Rev-mediated posttranscriptional regulation of human immunodeficiency virus type 1," J Virol. 71:95-101 (1997). . (see also my email message)

Takahashi, M.; Miyoshi, H.; Verma, I.M.; Gage, F.H., "Rescue from photoreceptor degeneration in the rd mouse by human immunodeficiency virus vector-mediated gene transfer," *J. Virol.* 73: 7812-7816 (Sept. 1999)

Uchida, N., Sutton, R.E., Friera, A.M., He, D., Reitsma, M.J., Chang, W.C., Veres, G., Scollay, R. & Weissman, I.L., "HIV, but not murine leukemia virus, vectors mediate high efficiency gene transfer into freshly isolated G0/G1 human hematopoietic stem cells," *Proc. Natl Acad. Sci. U S A.* 95, 11939-11944 (1998)

Valentin, A., W. Lu, M. Rosati, R. Schneider, J. Albert, A. Karlsson and G. N. Pavlakis. "Dual effect of interleukin 4 on HIV-1 expression: Implications for viral phenotypic switch and disease progression," *Proc. Natl Acad. Sci. U S A.* 95: 8886-91 (1998)

White, S.M., Renda, M, Nam, N-Y, Klimatcheva, E., Hu, Y, Fisk, J, Halterman, M, Rimel, B.J., Federoff, H, Pandya, S., Rosenblatt, J.D. and Planelles, V, "Lentivirus vectors using human and simian immunodeficiency virus elements," *J. Virol.* 73:2832-2840 (Apr. 1999)

Wolff, J.A. and Trubetskoy, V.S., "The Cambrian period of nonviral gene delivery," *Nature Biotechnology* 16:421-422 (1998)

Zolotukhin, J., Valentin, A., Pavlakis, G. N. and Felber, B. K. "Continuous propagation of RRE(-)and Rev(-)RRE(-) human immunodeficiency virus type 1 molecular clones containing a *cis*-acting element of Simian retrovirus type 1 in human peripheral blood lymphocytes," *J. Virol.* 68:7944-7952 (1994)

Zufferey, R., Nagy, D., Mandel, R.J., Naldini, L. and Trono, D., "Multiply Attenuated Lentiviral Vector Achieves Efficient Gene-Delivery In Vivo", *Nature Biotechnology* 15:871-875 (1997)

Zufferey, R., Dull, T., Mandel, R.J., Bukovsky, A., Quiroz, D., Naldini, L. & Trono, D., "Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery," *J. Virol.* 72:9873-9880 (1998)

Those skilled in the art will recognize that any gene encoding a mRNA containing an inhibitory/instability sequence or sequences can be modified in accordance with the exemplified methods of this invention or their functional equivalents.

Modifications of the above described modes for carrying out the invention that are obvious to those of skill in the fields of genetic engineering, virology, immunology, medicine, and related fields are intended to be within the scope of the following claims.

Every reference cited hereinbefore throughout the application is hereby incorporated by reference in its entirety.

WHAT IS CLAIMED IS:

1. A nucleic acid construct comprising a HIV-1 *gag/pol* gene having the coding sequence of the *gag/pol* gene set forth in Figure 1.

2. A nucleic acid construct comprising a HIV-1 *pol* gene having the coding sequence of the *pol* gene set forth in Figure 2.

3. A nucleic acid construct comprising a SIV-1 *gag* gene having the coding sequence of the *gag* gene set forth in Figure 3.

4. A nucleic acid construct comprising an HIV or SIV 5' LTR, a packaging signal, a *gag/pol* gene comprising the sequence set forth in Figure 1, a 5' splice site, a 3' splice site, an *env* gene, a *tat* gene, a functional RNA transport element and a 3' HIV or SIV LTR, said nucleic acid construct being able to produce functional Gag, Pol and Env virion components.

5. A vector comprising the nucleic acid construct of Claim 1, 2, 3 or 4.

6. A transformed host cell comprising the nucleic acid construct of Claim 1, 2, 3 or 4.

7. A transformed host cell of Claim 6 wherein said cell is a eukaryote.

8. The host cell of Claim 7 wherein said cell is a human cell.

9. A transformed host cell of Claim 6 wherein said cell is a prokaryote.

10. The host cell of Claim 9 wherein said cell is E. coli.

11. A pharmaceutical composition comprising the nucleic acid construct of Claim 1, 2, 3 or 4 and a pharmaceutically acceptable carrier.

12. A method for inducing antibodies in a mammal comprising administering to a mammal a composition of claim 11, wherein said nucleic acid construct is present in an amount which is effective to induce said antibodies in said mammal.

13. A method for inducing cytotoxic T lymphocytes in a mammal comprising administering to a mammal a composition of claim 11, wherein said nucleic acid construct is present in an amount which is effective to induce cytotoxic T lymphocytes in said mammal.

14. A vaccine composition for inducing immunity in a mammal against HIV infection comprising a pharmaceutically acceptable carrier and further comprising a therapeutically effective amount of a nucleic acid construct of Claim 1 capable of producing HIV Gag and Pol proteins in the absence of HIV Rev regulatory protein in a cell in vivo.

15. A vaccine composition for inducing immunity in a mammal against HIV infection comprising a pharmaceutically acceptable carrier and further comprising a therapeutically effective amount of a nucleic acid construct of Claim 2 capable of producing HIV Pol protein in the absence of HIV Rev regulatory protein in a cell in vivo.

16. A vaccine composition according to claim 14 wherein said mammal is a human.

17. A vaccine composition according to claim 15 wherein said mammal is a human.

18. A method for inducing immunity against HIV infection in a mammal which comprises administering to a mammal a therapeutically effective amount of a vaccine composition according to claim 14.

19. A method for inducing immunity against HIV infection in a mammal which comprises administering to a mammal a therapeutically effective amount of a vaccine composition according to claim 15.

20. A method according to claim 18 wherein said mammal is a human.

21. A method according to claim 19 wherein said mammal is a human.

22. A lentiviral expression system comprising the following:

(a) a packaging vector comprising a HIV-1 *gag/pol* gene having the nucleotide sequence set forth in Figure 1;

(b) a transfer vector; and

(c) an envelope encoding vector.

23. A transformed host cell comprising the lentiviral expression system of Claim 22.

24. A transformed host cell of Claim 23 wherein said cell is a eukaryote.

25. The host cell of Claim 24 wherein said cell is a human cell.

26. A process for making a lentiviral particle comprising expressing HIV Gag and HIV Pol in a host cell from a vector comprising the nucleotide sequences encoding HIV Gag and HIV Pol set forth in Figure 1 in the presence of a gene encoding an envelope protein.

27. A lentiviral expression system which is capable of functioning in the absence of Rev, Tat, and any viral RNA transport element comprising the following:

(a) a packaging vector comprising a HIV-1 *gag/pol* gene which has been mutated to eliminate inhibitory/instability regions;

(b) a transfer vector; and

(c) an envelope encoding vector.

28. A transformed host cell comprising the lentiviral expression system of Claim 27.

29. A transformed host cell of Claim 28 wherein said cell is a eukaryote.

30. The host cell of Claim 29 wherein said cell is a human cell.

31. A process for making a lentiviral particle in the absence of Rev, Tat, or any viral RNA transport element comprising expressing HIV Gag and HIV Pol in a host cell from a HIV-1 *gag/pol* gene which has been mutated to eliminate inhibitory/instability regions and expressing an Envelope protein from a envelope encoding gene whose expression is independent Rev, Tat, or any viral RNA transport element.

32. A nucleic acid construct comprising a SIV-1 *env* gene having the coding sequence of the *env* gene set forth in Figure 16.

33. A vector comprising the nucleic acid construct of claim 32.

34. A transformed host cell comprising the nucleic acid construct of claim 32.

35. A pharmaceutical composition comprising the nucleic acid construct of claim 32 and a pharmaceutically acceptable carrier.

36. A lentiviral expression system of claim 27 wherein the HIV-1 *gag/pol* gene has the coding sequence of the HIV-1 *gag/pol* gene set forth in Figure 1.

37. The process of claim 31 wherein the HIV-1 *gag/pol* gene has the coding sequence of the HIV-1 *gag/pol* gene set forth in Figure 1.

38. A lentiviral expression system of claim 27 wherein the packaging vector has the DNA sequence of packaging construct pCMVgag/polBNKan set forth in Figure 9.

39. A lentiviral expression system of claim 27 wherein the transfer vector has the DNA sequence of pmBCwCNluci set forth in Figure 10 or pmBCmCNluci set forth in Figure 11.

ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGATCGATGGGAAAAAAT

TCGGTTAAGGCCAGGGGGAAAGAAGAAGTACAAGCTAAAGCACATCGTATGGGCAA

GCAGGGAGCTAGAACGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGC

TGTAGACAAATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAGGAGCT

TCGATCACTATACAACACAGTAGCAACCCTCTATTGTGTGCACCAGCGGATCGAGA

TCAAGGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAGTCCAAG

AAGAAGGCCCAGCAGGCAGCAGCTGACACAGGACACAGCAATCAGGTCAGCCAAAA

TTACCCTATAGTGCAGAACATCCAGGGGCAAATGGTACATCAGGCCATATCACCTA

GAACTTTAAATGCATGGGTAAAAGTAGTAGAAGAGAAGGCTTTCAGCCCAGAAGTG

ATACCCATGTTTTCAGCATTATCAGAAGGAGCCACCCCACAGGACCTGAACACGAT

GTTGAACACCGTGGGGGGACATCAAGCAGCCATGCAAATGTTAAAAGAGACCATCA

ATGAGGAAGCTGCAGAATGGGATAGAGTGCATCCAGTGCATGCAGGGCCTATTGCA

CCAGGCCAGATGAGAGAACCAAGGGGAAGTGACATAGCAGGAACTACTAGTACCCT

TCAGGAACAAATAGGATGGATGACAAATAATCCACCTATCCCAGTAGGAGAGATCT

ACAAGAGGTGGATAATCCTGGGATTGAACAAGATCGTGAGGATGTATAGCCCTACC

AGCATTCTGGACATAAGACAAGGACCAAAGGAACCCTTTAGAGACTATGTAGACCG

GTTCTATAAAACTCTAAGAGCTGAGCAAGCTTCACAGGAGGTAAAAAATTGGATGA

CAGAAACCTTGTTGGTCCAAAATGCGAACCCAGATTGTAAGACCATCCTGAAGGCT

CTCGGCCCAGCGGCTACACTAGAAGAAATGATGACAGCATGTCAGGGAGTAGGAGG

ACCCGGCCATAAGGCAAGAGTTTTGGCCGAGGCGATGAGCCAGGTGACGAACTCGG

CGACCATAATGATGCAGAGAGGCAACTTCCGGAACCAGCGGAAGATCGTCAAGTGC

TTCAATTGTGGCAAAGAAGGGCACACCGCCAGGAACTGCCGGGCCCCCCGGAAGAA

GGGCTGTTGGAAATGTGGAAAGGAAGGACACCAAATGAAAGATTGTACTGAGAGAC

FIG. 1

AGGCTAATTTTTTAGGGAAGATCTGGCCTTCCTACAAGGGAAGGCCAGGGAATTTT

CTTCAGAGCAGACCAGAGCCAACAGCCCCACCAGAAGAGAGCTTCAGGTCTGGGGT

AGAGACAACAACTCCCCCTCAGAAGCAGGAGCCGATAGACAAGGAACTGTATCCTT

TAACTTCCCTCAGATCACTCTTTGGCAACGACCCCTCGTCACAGTAAGGATCGGGG

GGCAACTCAAGGAAGCGCTGCTCGATACAGGAGCAGATGATACAGTATTAGAAGAA

ATGAGTTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGGATCGGGGGCTTCAT

CAAGGTGAGGCAGTACGACCAGATACTCATAGAAATCTGTGGACATAAAGCTATAG

GTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAGAAATCTGTTGACC

CAGATCGGCTGCACCTTGAACTTCCCCATCAGCCCTATTGAGACGGTGCCCGTGAA

GTTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAATGGCCATTGACGAAAGAGA

AGATCAAGGCCTTAGTCGAAATCTGTACAGAGATGGAGAAGGAAGGGAAGATCAGC

AAGATCGGGCCTGAGAACCCCTACAACACTCCAGTCTTCGCAATCAAGAAGAAGGA

CAGTACCAAGTGGAGAAAGCTGGTGGACTTCAGAGAGCTGAACAAGAGAACTCAGG

ACTTCTGGGAAGTTCAGCTGGGCATCCCACATCCCGCTGGGTTGAAGAAGAAGAAG

TCAGTGACAGTGCTGGATGTGGGTGATGCCTACTTCTCCGTTCCCTTGGACGAGGA

CTTCAGGAAGTACACTGCCTTCACGATACCTAGCATCAACAACGAGACACCAGGCA

TCCGCTACCAGTACAACGTGCTGCCACAGGGATGGAAGGGATCACCAGCCATCTTT

CAAAGCAGCATGACCAAGATCCTGGAGCCCTTCCGCAAGCAAAACCCAGACATCGT

GATCTATCAGTACATGGACGACCTCTACGTAGGAAGTGACCTGGAGATCGGGCAGC

ACAGGACCAAGATCGAGGAGCTGAGACAGCATCTGTTGAGGTGGGGACTGACCACA

CCAGACAAGAAGCACCAGAAGGAACCTCCCTTCCTGTGGATGGGCTACGAACTGCA

TCCTGACAAGTGGACAGTGCAGCCCATCGTGCTGCCTGAGAAGGACAGCTGGACTG

TGAACGACATACAGAAGCTCGTGGGCAAGTTGAACTGGGCAAGCCAGATCTACCCA

GGCATCAAAGTTAGGCAGCTGTGCAAGCTGCTTCGAGGAACCAAGGCACTGACAGA

AGTGATCCCACTGACAGAGGAAGCAGAGCTAGAACTGGCAGAGAACCGAGAGATCC

TGAAGGAGCCAGTACATGGAGTGTACTACGACCCAAGCAAGGACCTGATCGCAGAG

ATCCAGAAGCAGGGGCAAGGCCAATGGACCTACCAAATCTACCAGGAGCCCTTCAA

GAACCTGAAGACAGGCAAGTACGCAAGGATGAGGGGTGCCCACACCAACGATGTGA

AGCAGCTGACAGAGGCAGTGCAGAAGATCACCACAGAGAGCATCGTGATCTGGGGC

AAGACTCCCAAGTTCAAGCTGCCCATACAGAAGGAGACATGGGAGACATGGTGGAC

CGAGTACTGGCAAGCCACCTGGATCCCTGAGTGGGAGTTCGTGAACACCCCTCCCT

TGGTGAAACTGTGGTATCAGCTGGAGAAGGAACCCATCGTGGGAGCAGAGACCTTC

TACGTGGATGGGGCAGCCAACAGGGAGACCAAGCTGGGCAAGGCAGGCTACGTGAC

CAACCGAGGACGACAGAAAGTGGTGACCCTGACTGACACCACCAACCAGAAGACTG

AGCTGCAAGCCATCTACCTAGCTCTGCAAGACAGCGGACTGGAAGTGAACATCGTG

ACAGACTCACAGTACGCACTGGGCATCATCCAAGCACAACCAGACCAATCCGAGTC

AGAGCTGGTGAACCAGATCATCGAGCAGCTGATCAAGAAGGAGAAAGTGTACCTGG

CATGGGTACCAGCACACAAAGGAATTGGAGGAAATGAACAAGTAGATAAATTAGTC

AGTGCTGGGATCCGGAAGGTGCTGTTCCTGGACGGGATCGATAAGGCCCAAGATGA

ACATGAGAAGTACCACTCCAACTGGCGCGCTATGGCCAGCGACTTCAACCTGCCAC

CTGTAGTAGCAAAAGAAATAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGGAGAA

GCCATGCATGGACAAGTAGACTGTAGTCCAGGAATATGGCAGCTGGACTGCACGCA

CCTGGAGGGGAAGGTGATCCTGGTAGCAGTTCATGTAGCCAGTGGATATATAGAAG

CAGAAGTTATCCCTGCTGAAACTGGGCAGGAAACAGCATATTTTCTTTTAAAATTA

GCAGGAAGATGGCCAGTAAAAACAATACACACGGACAACGGAAGCAACTTCACTGG

TGCTACGGTTAAGGCCGCCTGTTGGTGGGCGGGAATCAAGCAGGAATTTGGAATTC

CCTACAATCCCCAATCGCAAGGAGTCGTGGAGAGCATGAACAAGGAGCTGAAGAAG

ATCATCGGACAAGTGAGGGATCAGGCTGAGCACCTGAAGACAGCAGTGCAGATGGC

AGTGTTCATCCACAACTTCAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGG

AAAGGATCGTGGACATCATCGCCACCGACATCCAAACCAAGGAGCTGCAGAAGCAG

ATCACCAAGATCCAGAACTTCCGGGTGTACTACCGCGACAGCCGCAACCCACTGTG

GAAGGGACCAGCAAAGCTCCTCTGGAAGGGAGAGGGGGCAGTGGTGATCCAGGACA

ACAGTGACATCAAAGTGGTGCCAAGGCGCAAGGCCAAGATCATCCGCGACTATGGA

AAACAGATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAGAACCT

GGAAGAGCCTGGTGAAGCACCATATG (SEQUENCE ID NO:1)

```
>wildtype    TGTACAGAGA TGGAAAAGGA AGGGAAAATT TCAAAAATTG
>mutated     TGTACAGAGA TGGAGAAGGA AGGGAAGATC AGCAAGATCG
 #1          ..........................................
                       *           * * *** * *

>wildtype    GGCCTGAAAA TCCATACAAT ACTCCAGTAT TTGCCATAAA
>mutated     GGCCTGAGAA CCCCTACAAC ACTCCAGTCT TCGCAATCAA
 #41         ..........................................
                  * * *    *        *  * * *

>wildtype    GAAAAAAGAC AGTACTAAAT GGAGAAAATT AGTAGATTTC
>mutated     GAAGAAGGAC AGTACCAAGT GGAGAAAGCT GGTGGACTTC
 #81         ..........................................
               * *       * *       ** * * *

>wildtype    AGAGAACTTA ATAAGAGAAC TCAAGACTTC TGGGAAGTTC
>mutated     AGAGAGCTGA ACAAGAGAAC TCAGGACTTC TGGGAAGTTC
 #121        ..........................................
                * * *          *

>wildtype    AATTAGGAAT ACCACATCCC GCAGGGTTAA AAAAGAAAAA
>mutated     AGCTGGGCAT CCCACATCCC GCTGGGTTGA AGAAGAAGAA
 #161        ..........................................
              ** * *  *          *    *  *    *

>wildtype    ATCAGTAACA GTACTGGATG TGGGTGATGC ATATTTTTCA
>mutated     GTCAGTGACA GTGCTGGATG TGGGTGATGC CTACTTCTCC
 #201        ..........................................
             *    *      *                 * * * *

>wildtype    GTTCCCTTAG ATGAAGACTT CAGGAAATAT ACTGCATTTA
>mutated     GTTCCCTTGG ACGAGGACTT CAGGAAGTAC ACTGCCTTCA
 #241        ..........................................
                  * * *           * *     * *

>wildtype    CCATACCTAG TATAAACAAT GAGACACCAG GGATTAGATA
>mutated     CGATACCTAG CATCAACAAC GAGACACCAG GCATCCGCTA
 #281        ..........................................
              *       * *   *             * ** *

>wildtype    TCAGTACAAT GTGCTTCCAC AGGGATGGAA AGGATCACCA
>mutated     CCAGTACAAC GTGCTGCCAC AGGGATGGAA GGGATCACCA
 #321        ..........................................
             *       *     *               *

>wildtype    GCAATATTCC AAAGTAGCAT GACAAAAATC TTAGAGCCTT
>mutated     GCCATCTTTC AAAGCAGCAT GACCAAGATC CTGGAGCCCT
 #361        ..........................................
               * * *     *       * *   * *    *

>wildtype    TTAGAAAACA AAATCCAGAC ATAGTTATCT ATCAATACAT
>mutated     TCCGCAAGCA AAACCCAGAC ATCGTGATCT ATCAGTACAT
 #401        ..........................................
              ** * *     *       * *       *
```

FIG. 2

```
>wildtype       GGATGATTTG TATGTAGGAT CTGACTTAGA AATAGGGCAG
>mutated        GGACGACCTC TACGTAGGAA GTGACCTGGA GATCGGGCAG
 #441           ..........................................
                   *  ** *   *      * *    * *   *  *

>wildtype       CATAGAACAA AAATAGAGGA GCTGAGACAA CATCTGTTGA
>mutated        CACAGGACCA AGATCGAGGA GCTGAGACAG CATCTGTTGA
 #481           ..........................................
                  *  *  *   *  *              *

>wildtype       GGTGGGGACT TACCACACCA GACAAAAAAC ATCAGAAAGA
>mutated        GGTGGGGACT GACCACACCA GACAAGAAGC ACCAGAAGGA
 #521           ..........................................
                          *                 *  *   *    *

>wildtype       ACCTCCATTC CTTTGGATGG GTTATGAACT CCATCCTGAT
>mutated        ACCTCCCTTC CTGTGGATGG GCTACGAACT GCATCCTGAC
 #561           ..........................................
                     *     *         *  *      *        *

>wildtype       AAATGGACAG TACAGCCTAT AGTGCTGCCA GAAAAAGACA
>mutated        AAGTGGACAG TGCAGCCCAT CGTGCTGCCT GAGAAGGACA
 #601           ..........................................
                  *        *    *   *        *   *  *

>wildtype       GCTGGACTGT CAATGACATA CAGAAGTTAG TGGGGAAATT
>mutated        GCTGGACTGT GAACGACATA CAGAAGCTCG TGGGCAAGTT
 #641           ..........................................
                        *  *            * *     *  *

>wildtype       GAATTGGGCA AGTCAGATTT ACCCAGGGAT TAAAGTAAGG
>mutated        GAACTGGGCA AGCCAGATCT ACCCAGGCAT CAAAGTTAGG
 #681           ..........................................
                   *        *    *        *   *    *

>wildtype       CAATTATGTA AACTCCTTAG AGGAACCAAA GCACTAACAG
>mutated        CAGCTGTGCA AGCTGCTTCG AGGAACCAAG GCACTGACAG
 #721           ..........................................
                  ** *  *   *  *  *          *      *

>wildtype       AAGTAATACC ACTAACAGAA GAAGCAGAGC TAGAACTGGC
>mutated        AAGTGATCCC ACTGACAGAG GAAGCAGAGC TAGAACTGGC
 #761           ..........................................
                    *  *     *    *

>wildtype       AGAAAACAGA GAGATTCTAA AAGAACCAGT ACATGGAGTG
>mutated        AGAGAACCGA GAGATCCTGA AGGAGCCAGT ACATGGAGTG
 #801           ..........................................
                   *  *        *  *   *  *

>wildtype       TATTATGACC CATCAAAAGA CTTAATAGCA GAAATACAGA
>mutated        TACTACGACC CAAGCAAGGA CCTGATCGCA GAGATCCAGA
 #841           ..........................................
                  *  *       ***  *    * *  *      *  *
```

```
>wildtype      AGCAGGGGCA AGGCCAATGG ACATATCAAA TTTATCAAGA
>mutated       AGCAGGGGCA AGGCCAATGG ACCTACCAAA TCTACCAGGA
 #881          ..........................................
                                     *  *     *  *  *

>wildtype      GCCATTTAAA AATCTGAAAA CAGGAAAATA TGCAAGAATG
>mutated       GCCCTTCAAG AACCTGAAGA CAGGCAAGTA CGCAAGGATG
 #921          ..........................................
                  *  *  *  *     *     *  *  *     *

>wildtype      AGGGGTGCCC ACACTAATGA TGTAAAACAA TTAACAGAGG
>mutated       AGGGGTGCCC ACACCAACGA TGTGAAGCAG CTGACAGAGG
 #961          ..........................................
                           *  *     *  *  *  *  *

>wildtype      CAGTGCAAAA AATAACCACA GAAAGCATAG TAATATGGGG
>mutated       CAGTGCAGAA GATCACCACA GAGAGCATCG TGATCTGGGG
 #1001         ..........................................
                     *  *  *        *     *  *  *

>wildtype      AAAGACTCCT AAATTTAAAC TGCCCATACA AAAGGAAACA
>mutated       CAAGACTCCC AAGTTCAAGC TGCCCATACA GAAGGAGACA
 #1041         ..........................................
               *        *  *  *  *              *     *

>wildtype      TGGGAAACAT GGTGGACAGA GTATTGGCAA GCCACCTGGA
>mutated       TGGGAGACAT GGTGGACCGA GTACTGGCAA GCCACCTGGA
 #1081         ..........................................
                    *           *     *

>wildtype      TTCCTGAGTG GGAGTTTGTT AATACCCCTC CTTTAGTGAA
>mutated       TCCCTGAGTG GGAGTTCGTG AACACCCCTC CCTTGGTGAA
 #1121         ..........................................
                *              *  *  *        *  *

>wildtype      ATTATGGTAC CAGTTAGAGA AAGAACCCAT AGTAGGAGCA
>mutated       ACTGTGGTAT CAGCTGGAGA AGGAACCCAT CGTGGGAGCA
 #1161         ..........................................
                * *    *    * *     *        *  *

>wildtype      GAAACCTTCT ATGTAGATGG GGCAGCTAAC AGGGAGACTA
>mutated       GAGACCTTCT ACGTGGATGG GGCAGCCAAC AGGGAGACCA
 #1201         ..........................................
                *        *  *              *           *

>wildtype      AATTAGGAAA AGCAGGATAT GTTACTAATA GAGGAAGACA
>mutated       AGCTGGGCAA GGCAGGCTAC GTGACCAACC GAGGACGACA
 #1241         ..........................................
                ** *  *   *     *  *   *  *  **      *

>wildtype      AAAAGTTGTC ACCCTAACTG ACACAACAAA TCAGAAGACT
>mutated       GAAAGTGGTG ACCCTGACTG ACACCACCAA CCAGAAGACT
 #1281         ..........................................
               *   *  *      *         *  *   *
```

```
>wildtype      GAGTTACAAG CAATTTATCT AGCTTTGCAG GATTCGGGAT
>mutated       GAGCTGCAAG CCATCTACCT AGCTCTGCAA GACAGCGGAC
 #1321         ..........................................
                 * *     * * *       *   *  ****  *

>wildtype      TAGAAGTAAA CATAGTAACA GACTCACAAT ATGCATTAGG
>mutated       TGGAAGTGAA CATCGTGACA GACTCACAGT ACGCACTGGG
 #1361         ..........................................
                *    *     * *           *   *  * *

>wildtype      AATCATTCAA GCACAACCAG ATCAAAGTGA ATCAGAGTTA
>mutated       CATCATCCAA GCACAACCAG ACCAATCCGA GTCAGAGCTG
 #1401         ..........................................
               *    *               *  ***  *     * *

>wildtype      GTCAATCAAA TAATAGAGCA GTTAATAAAA AAGGAAAAGG
>mutated       GTGAACCAGA TCATCGAGCA GCTGATCAAG AAGGAGAAAG
 #1441         ..........................................
                 * * *   * *     * * *  *     * *

>wildtype      TCTATCTGGC ATGGGTACCA GCACACAAAG GAATTGGAGG
>mutated       TGTACCTGGC ATGGGTACCA GCACACAAAG GAATTGGAGG
 #1481         ..........................................
                * *

>wildtype      AAATGAACAA GTAGATAAAT TAGTCAGTGC TGGAATCAGG
>mutated       AAATGAACAA GTAGATAAAT TAGTCAGTGC TGGGATCCGG
 #1521         ..........................................
                                            *  *

>wildtype      AAAGTACTAT TTTTAGATGG AATAGATAAG GCCCAAGATG
>mutated       AAGGTGCTGT TCCTGGACGG GATCGATAAG GCCCAAGATG
 #1561         ..........................................
                 * * *  ** * *  * *

>wildtype      AACATGAGAA ATATCACAGT AATTGGAGAG CAATGGCTAG
>mutated       AACATGAGAA GTACCACTCC AACTGGCGCG CTATGGCCAG
 #1601         ..........................................
                       * *  ***  *  * *  *    *

>wildtype      TGATTTTAAC CTGCCACCTG TAGTAGCAAA AGAAATAGTA
>mutated       CGACTTCAAC CTGCCACCTG TAGTAGCAAA AGAAATAGTA
 #1641         ..........................................
               * * *

>wildtype      GCCAGCTGTG ATAAATGTCA GCTAAAAGGA GAAGCCATGC
>mutated       GCCAGCTGTG ATAAATGTCA GCTAAAAGGA GAAGCCATGC
 #1681         ..........................................

>wildtype      ATGGACAAGT AGACTGTAGT CCAGGAATAT GGCAACTAGA
>mutated       ATGGACAAGT AGACTGTAGT CCAGGAATAT GGCAGCTGGA
 #1721         ..........................................
                                              * *
```

```
>wildtype     TTGTACACAT TTAGAAGGAA AAGTTATCCT GGTAGCAGTT
>mutated      CTGCACGCAC CTGGAGGGGA AGGTGATCCT GGTAGCAGTT
 #1761        ..........................................
              *  *  *  * * *  *  *   *  *

>wildtype     CATGTAGCCA GTGGATATAT AGAAGCAGAA GTTATTCCAG
>mutated      CATGTAGCCA GTGGATATAT AGAAGCAGAA GTTATCCCTG
 #1801        ..........................................
                                                 *  *

>wildtype     CAGAAACAGG GCAGGAAACA GCATATTTTC TTTTAAAATT
>mutated      CTGAAACTGG GCAGGAAACA GCATATTTTC TTTTAAAATT
 #1841        ..........................................
               *     *

>wildtype     AGCAGGAAGA TGGCCAGTAA AAACAATACA TACAGACAAT
>mutated      AGCAGGAAGA TGGCCAGTAA AAACAATACA CACGGACAAC
 #1881        ..........................................
                                             *  *     *

>wildtype     GGCAGCAATT TCACCAGTGC TACGGTTAAG GCCGCCTGTT
>mutated      GGAAGCAACT TCACTGGTGC TACGGTTAAG GCCGCCTGTT
 #1921        ..........................................
                *     *      **

>wildtype     GGTGGGCGGG AATCAAGCAG GAATTTGGAA TTCCCTACAA
>mutated      GGTGGGCGGG AATCAAGCAG GAATTTGGAA TTCCCTACAA
 #1961        ..........................................

>wildtype     TCCCCAAAGT CAAGGAGTAG TAGAATCTAT GAATAAAGAA
>mutated      TCCCCAATCG CAAGGAGTCG TGGAGAGCAT GAACAAGGAG
 #2001        ..........................................
                    ***         *   *  ****     *  *  *

>wildtype     TTAAAGAAAA TTATAGGACA GGTAAGAGAT CAGGCTGAAC
>mutated      CTGAAGAAGA TCATCGGACA AGTGAGGGAT CAGGCTGAGC
 #2041        ..........................................
              * *     *   *  *      *  *  *           *

>wildtype     ATCTTAAGAC AGCAGTACAA ATGGCAGTAT TCATCCACAA
>mutated      ACCTGAAGAC AGCAGTGCAG ATGGCAGTGT TCATCCACAA
 #2081        ..........................................
               *  *           *  *         *

>wildtype     TTTTAAAAGA AAAGGGGGGA TTGGGGGGTA CAGTGCAGGG
>mutated      CTTCAAAAGA AAAGGGGGGA TTGGGGGGTA CAGTGCAGGG
 #2121        ..........................................
              *  *

>wildtype     GAAAGAATAG TAGACATAAT AGCAACAGAC ATACAAACTA
>mutated      GAAAGGATCG TGGACATCAT CGCCACCGAC ATCCAAACCA
 #2161        ..........................................
                   *  *   *    *   *  *  *     *    *
```

```
>wildtype      AAGAATTACA AAAACAAATT ACAAAAATTC AAAATTTTCG
>mutated       AGGAGCTGCA GAAGCAGATC ACCAAGATCC AGAACTTCCG
 #2201         ..........................................
                *  ** *   *  *  *  *   *  *  *   *  *  *

>wildtype      GGTTTATTAC AGGGACAGCA GAAATCCACT TTGGAAAGGA
>mutated       GGTGTACTAC CGCGACAGCC GCAACCCACT GTGGAAGGGA
 #2241         ..........................................
                  *  *    * *      *  *  *      *     *

>wildtype      CCAGCAAAGC TCCTCTGGAA AGGTGAAGGG GCAGTAGTAA
>mutated       CCAGCAAAGC TCCTCTGGAA GGGAGAGGGG GCAGTGGTGA
 #2281         ..........................................
                                  *  *  *         *  *

>wildtype      TACAAGATAA TAGTGACATA AAAGTAGTGC CAAGAAGAAA
>mutated       TCCAGGACAA CAGTGACATC AAAGTGGTGC CAAGGCGCAA
 #2321         ..........................................
                *  *  *   *        *       *         ** *

>wildtype      AGCAAAGATC ATTAGGGATT ATGGAAAACA GATGGCAGGT
>mutated       GGCCAAGATC ATCCGCGACT ATGGAAAACA GATGGCAGGT
 #2361         ..........................................
               *  *         ** *  *

>wildtype      GATGATTGTG TGGCAAGTAG ACAGGATGAG GATTAGAACA
>mutated       GATGATTGTG TGGCAAGTAG ACAGGATGAG GATTAGAACC
 #2401         ..........................................
                                                       *

>wildtype      TGGAAAAGTT TAGTAAAACA CCATATG
>mutated       TGGAAGAGCC TGGTGAAGCA CCATATG
 #2441         ..........................................
                    *  **  *  *  *
```

ATGGGCGTGAGAAACTCCGTCTTGTCAGGGAAGAAAGCAGATGAATTAG
AAAAAATTAGGCTACGACCCAACGGAAAGAAAAAGTACATGTTGAAGC
ATGTAGTATGGGCAGCAAATGAATTAGATAGATTTGGATTAGCAGAAAG
CCTGTTGGAGAACAAAGAAGGATGTCAAAAAATACTTTCGGTCTTAGCT
CCATTAGTGCCAACAGGCTCAGAAAATTTAAAAAGCCTTTATAATACTG
TCTGCGTCATCTGGTGCATTCACGCAGAAGAGAAAGTGAAACACACTGA
GGAAGCAAAACAGATAGTGCAGAGACACCTAGTGGTGGAAACAGGAAC
CACCGAAACCATGCCGAAGACCTCTCGACCAACAGCACCATCTAGCGGC
AGAGGAGGAAACTACCCAGTACAGCAGATCGGTGGCAACTACGTCCAC
CTGCCACTGTCCCCGAGAACCCTGAACGCTTGGGTCAAGCTGATCGAGG
AGAAGAAGTTCGGAGCAGAAGTAGTGCCAGGATTCCAGGCACTGTCAG
AAGGTTGCACCCCCTACGACATCAACCAGATGCTGAACTGCGTTGGAGA
CCATCAGGCGGCTATGCAGATCATCCGTGACATCATCAACGAGGAGGCT
GCAGATTGGGACTTGCAGCACCCACAACCAGCTCCACAACAAGGACAA
CTTAGGGAGCCGTCAGGATCAGACATCGCAGGAACCACCTCCTCAGTTG
ACGAACAGATCCAGTGGATGTACCGTCAGCAGAACCCGATCCCAGTAGG
CAACATCTACCGTCGATGGATCCAGCTGGGTCTGCAGAAATGCGTCCGT
ATGTACAACCCGACCAACATTCTAGATGTAAAACAAGGGCCAAAAGAG
CCATTTCAGAGCTATGTAGACAGGTTCTACAAAAGTTTAAGAGCAGAAC
AGACAGATGCAGCAGTAAAGAATTGGATGACTCAAACACTGCTGATTCA
AAATGCTAACCCAGATTGCAAGCTAGTGCTGAAGGGGCTGGGTGTGAAT
CCCACCCTAGAAGAAATGCTGACGGCTTGTCAAGGAGTAGGGGGGCCG
GGACAGAAGGCTAGATTAATGGCAGAAGCCCTGAAAGAGGCCCTCGCA
CCAGTGCCAATCCCTTTTGCAGCAGCCCAACAGAGGGGACCAAGAAAGC
CAATTAAGTGTTGGAATTGTGGGAAAGAGGGACACTCTGCAAGGCAATG
CAGAGCCCCAAGAAGACAGGGATGCTGGAAATGTGGAAAAATGGACCA
TGTTATGGCCAAATGCCCAGACAGACAGGCGGGTTTTTTAGGCCTTGGT
CCATGGGGAAAGAAGCCCCGCAATTTCCCCATGGCTCAAGTGCATCAGG
GGCTGATGCCAACTGCTCCCCCAGAGGACCCAGCTGTGGATCTGCTAAA
GAACTACATGCAGTTGGGCAAGCAGCAGAGAGAAAAGCAGAGAGAAAG
CAGAGAGAAGCCTTACAAGGAGGTGACAGAGGATTTGCTGCACCTCAAT
TCTCTCTTTGGAGGAGACCAGTAG

FIG. 3

```
SIV gag   ----------------------------------------
 #1        ........................................
          ATGGGCGTGAGAAACTCCGTCTTGTCAGGGAAGAAAGCAG

SIV gag   ----------------------------------------
 #41       ........................................
          ATGAATTAGAAAAAATTAGGCTACGACCCAACGGAAAGAA

SIV gag   ----------------------------------------
 #81       ........................................
          AAAGTACATGTTGAAGCATGTAGTATGGGCAGCAAATGAA

SIV gag   ----------------------------------------
 #121      ........................................
          TTAGATAGATTTGGATTAGCAGAAAGCCTGTTGGAGAACA

SIV gag   ----------------------------------------
 #161      ........................................
          AAGAAGGATGTCAAAAAATACTTTCGGTCTTAGCTCCATT

SIV gag   ----------------------------------------
 #201      ........................................
          AGTGCCAACAGGCTCAGAAAATTTAAAAAGCCTTTATAAT

SIV gag   ----------------------------------------
 #241      ........................................
          ACTGTCTGCGTCATCTGGTGCATTCACGCAGAAGAGAAAG

SIV gag   ----------------------------------------
SIVgagDX..                                ----------
 #281      ........................................
          TGAAACACACTGAGGAAGCAAAACAGATAGTGCAGAGACA

SIV gag   --------------------A--A-----T-----A--A
SIVgagDX..--------------------C--C-----C-----G--G
 #321      ........................................
          CCTAGTGGTGGAAACAGGAACMACMGAAACYATGCCRAAR

SIV gag   --AAG-A-----------------------------------
SIVgagDX..--CTC-C-----------------------------------
 #361      ........................................
          ACMWSTMGACCAACAGCACCATCTAGCGGCAGAGGAGGAA
```

FIG. 4

```
SIV gag   -T----------A--A--A----------T--------
SIVgagDX..-C----------G--G--C----------C--------
 #401      ....................................
           AYTACCCAGTACARCARATMGGTGGTAACTAYGTCCACCT

SIV gag   ----T-AAG---------AT-A--T--C-----A--AT--
SIVgagDX..----C-GTC---------CC-G--C--T-----C--GC--
 #441      ....................................
           GCCAYTRWSCCCGAGAACMYTRAAYGCYTGGGTMAARYTG

SIV gag   --A-----A-----A--T--------------------
SIVgagDX..--C-----G-----G--C--------------------
 #481      ....................................
           ATMGAGGARAAGAARTTYGGAGCAGAAGTAGTGCCAGGAT

SIV gag   -T----------------------------T-----T--
SIVgagDX..-C----------------------------C-----C--
 #521      ....................................
           TYCAGGCACTGTCAGAAGGTTGCACCCCCTAYGACATYAA

SIV gag   T------T-A--T--T--G----------A---------
SIVgagDX..C------C-G--C--C--T----------G---------
 #561      ....................................
           YCAGATGYTRAAYTGYGTKGGAGACCATCARGCGGCTATG

SIV gag   -----T---A-A--T--T--A------------------
SIVgagDX..-----C---C-T--C--C--C------------------
 #601      ....................................
           CAGATYATCMGWGAYATYATMAACGAGGAGGCTGCAGATT

SIV gag   ------------------------------------
SIVgagDX..------------------------------------
 #641      ....................................
           GGGACTTGCAGCACCCACAACCAGCTCCACAACAAGGACA

SIV gag   ----------------------T--T--------A--T
SIVgagDX..----------------------C--C--------C--C
 #681      ....................................
           ACTTAGGGAGCCGTCAGGATCAGAYATYGCAGGAACMACY

SIV gag   AGT-----A--T-----A---------------A-A--A-
SIVgagDX..TCC-----T--C-----G---------------C-T--G-
 #721      ....................................
           WSYTCAGTWGAYGAACARATCCAGTGGATGTACMGWCARC
```

```
SIV gag    -------C--A--------------T---A-GA-------
SIVgagDX..-------G--C--------------C---C-TC-------
 #761       ......................................
           AGAACCCSATMCCAGTAGGCAACATYTACMGKMGATGGAT

SIV gag    ---A-----GT----A--A--T--CA-A-----T-----A
SIVgagDX..---G-----TC----G--G--C--TC-T-----C-----G
 #801       ......................................
           CCARCTGGGKYTGCARAARTGYGTYMGWATGTAYAACCCR

SIV gag    --A-----------------------------------
SIVgagDX..--C-------------------
 #841       ......................................
           ACMAACATTCTAGATGTAAAACAAGGGCCAAAAGAGCCAT

SIV gag    ---------------------------------------
 #881       ......................................
           TTCAGAGCTATGTAGACAGGTTCTACAAAAGTTTAAGAGC

SIV gag    ---------------------------------------
 #921       ......................................
           AGAACAGACAGATGCAGCAGTAAAGAATTGGATGACTCAA

SIV gag    ---------------------------------------
 #961       ......................................
           ACACTGCTGATTCAAAATGCTAACCCAGATTGCAAGCTAG

SIV gag    ---------------------------------------
 #1001      ......................................
           TGCTGAAGGGGCTGGGTGTGAATCCCACCCTAGAAGAAAT

SIV gag    ---------------------------------------
 #1041      ......................................
           GCTGACGGCTTGTCAAGGAGTAGGGGGGCCGGGACAGAAG

SIV gag    ---------------------------------------
 #1081      ......................................
           GCTAGATTAATGGCAGAAGCCCTGAAAGAGGCCCTCGCAC

SIV gag    ---------------------------------------
 #1121      ......................................
           CAGTGCCAATCCCTTTTGCAGCAGCCCAACAGAGGGGACC

SIV gag    ---------------------------------------
 #1161      ......................................
           AAGAAAGCCAATTAAGTGTTGGAATTGTGGGAAAGAGGGA
```

```
SIV gag  ----------------------------------------
#1201    ........................................
         CACTCTGCAAGGCAATGCAGAGCCCCAAGAAGACAGGGAT

SIV gag  ----------------------------------------
#1241    ........................................
         GCTGGAAATGTGGAAAAATGGACCATGTTATGGCCAAATG

SIV gag  ----------------------------------------
#1281    ........................................
         CCCAGACAGACAGGCGGGTTTTTTAGGCCTTGGTCCATGG

SIV gag  ----------------------------------------
#1321    ........................................
         GGAAAGAAGCCCCGCAATTTCCCCATGGCTCAAGTGCATC

SIV gag  ----------------------------------------
#1361    ........................................
         AGGGGCTGATGCCAACTGCTCCCCCAGAGGACCCAGCTGT

SIV gag  ----------------------------------------
#1401    ........................................
         GGATCTGCTAAAGAACTACATGCAGTTGGGCAAGCAGCAG

SIV gag  ----------------------------------------
#1441    ........................................
         AGAGAAAAGCAGAGAGAAAGCAGAGAGAAGCCTTACAAGG

SIV gag  ----------------------------------------
#1481    ........................................
         AGGTGACAGAGGATTTGCTGCACCTCAATTCTCTCTTTGG

SIV gag  ----------------------------------------
#1521    ........................................
         AGGAGACCAGTAG
```

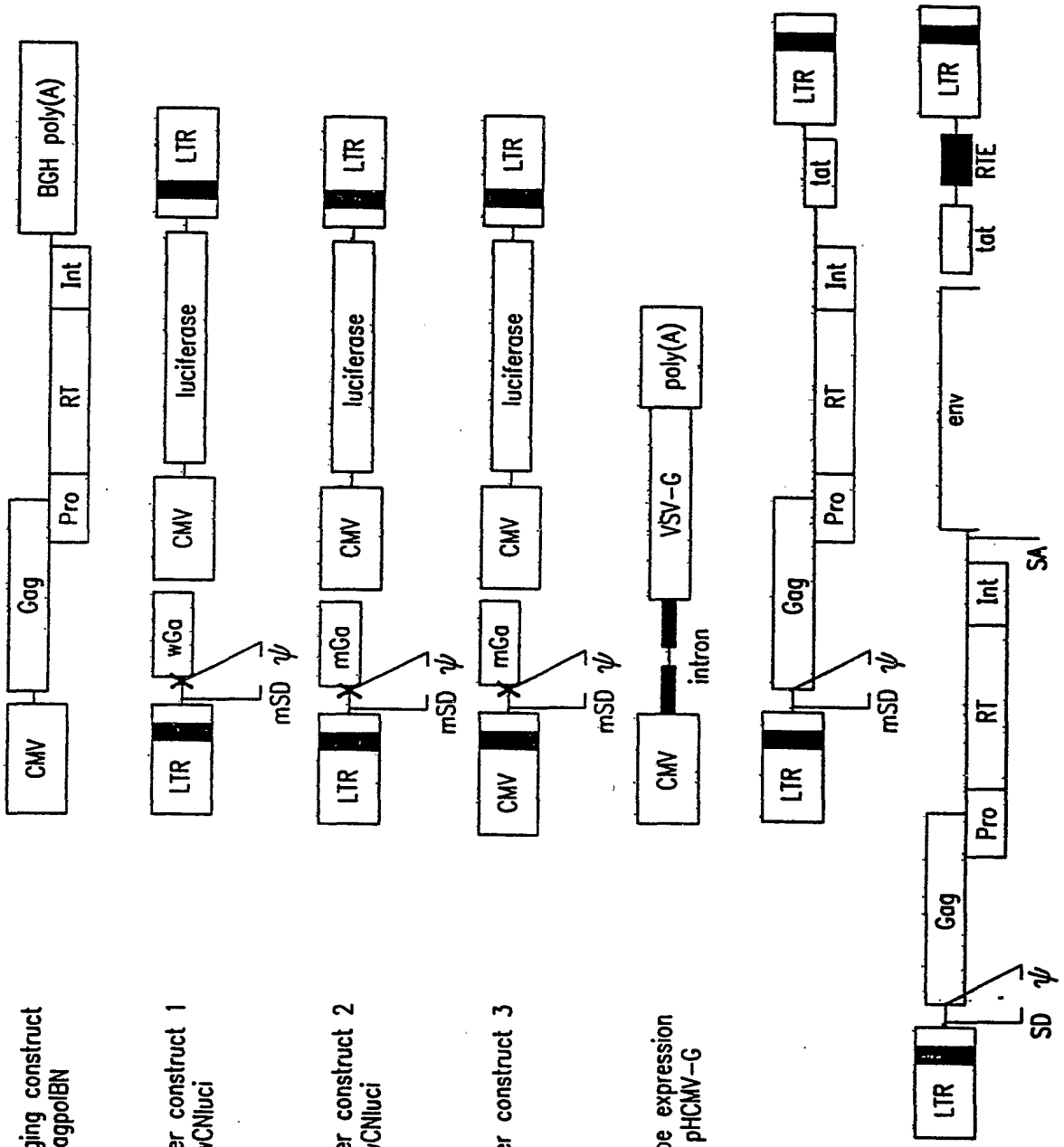

Packaging construct pCMVgagpolBN
CMV
Gag
Pro
RT
Int
BGH poly(A)
Transfer construct 1 pmBCwCNluci
LTR
wGa
mSD
ψ
CMV
luciferase
LTR
Transfer construct 2 pmBCwCNluci
LTR
mGa
mSD
ψ
CMV
luciferase
LTR
Transfer construct 3
CMV
mGa
mSD
ψ
CMV
luciferase
LTR
Envelope expression vector pHCMV-G
CMV
intron
VSV-G
poly(A)
MV1
LTR
Gag
Pro
RT
Int
tat
LTR
mSD
ψ
LTR
MV2
LTR
Gag
Pro
RT
Int
SA
env
tat
RTE
LTR
SD
ψ

FIG. 5

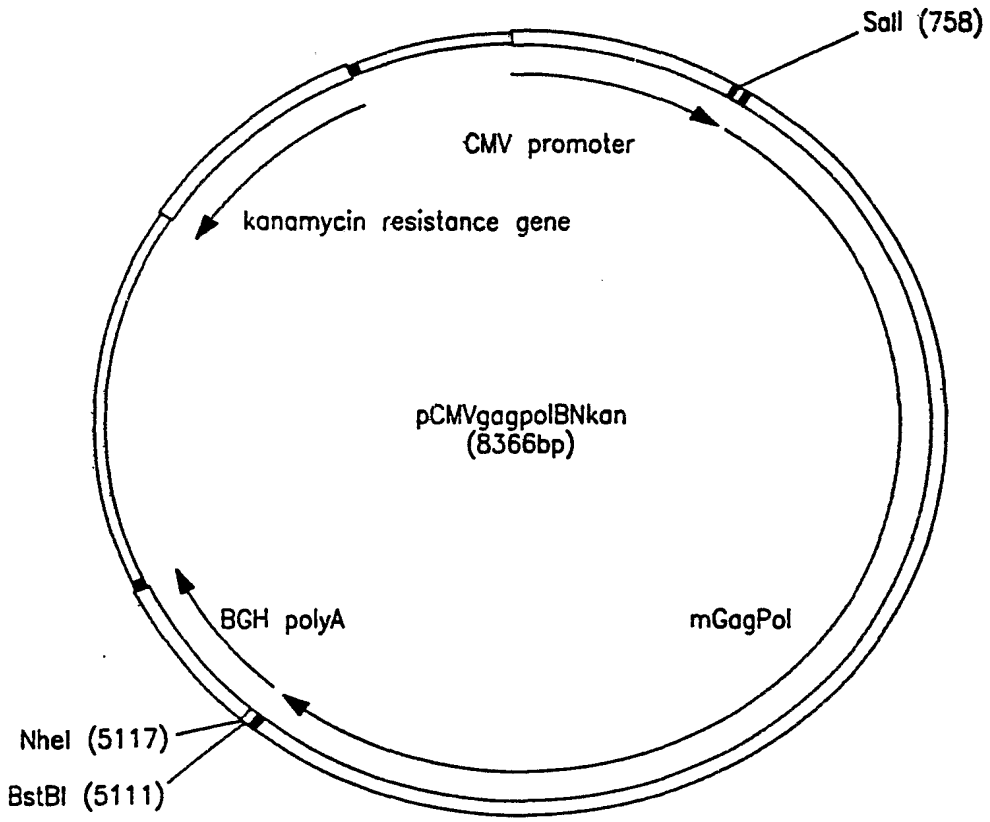

SalI (758)
CMV promoter
kanamycin resistance gene
pCMVgagpolBNkan
(8366bp)
BGH polyA
mGagPol
NheI (5117)
BstBI (5111)

FIG. 6

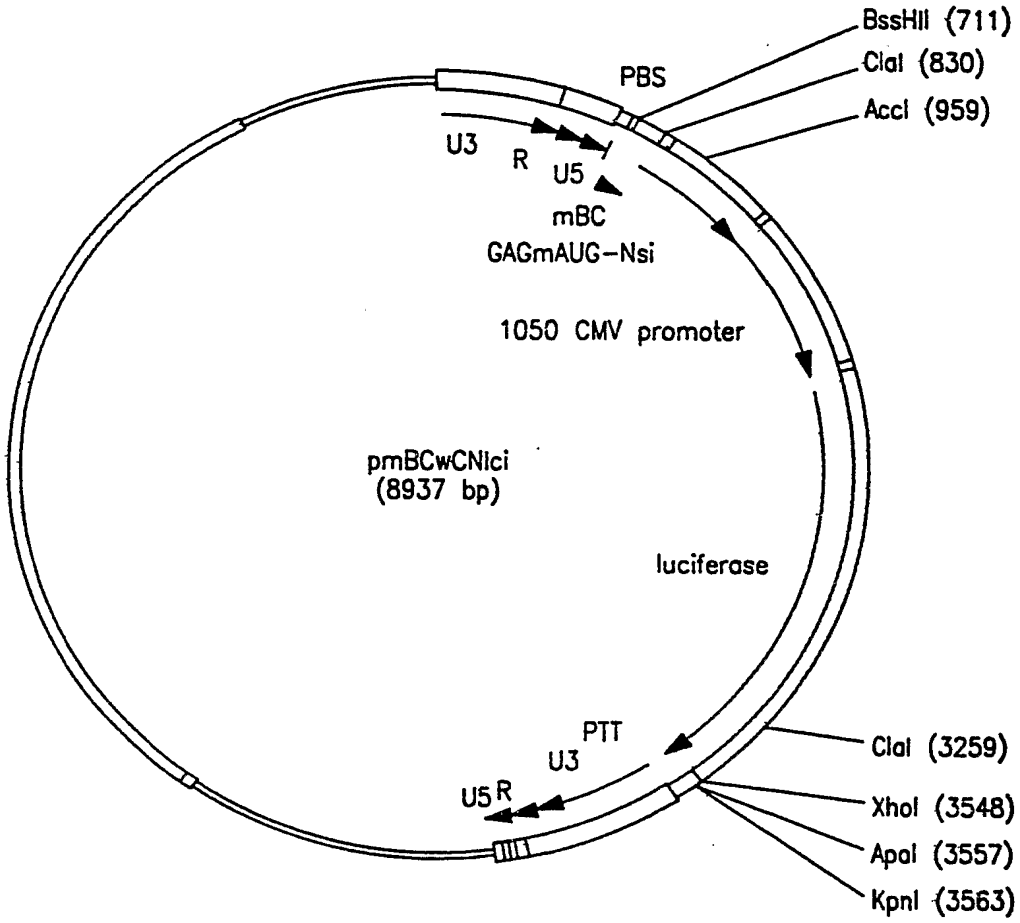

BssHII (711)
ClaI (830)
AccI (959)
PBS
U3
R
U5
mBC
GAGmAUG–NsiI
1050 CMV promoter
pmBCwCNIci
(8937 bp)
luciferase
PTT
U3
U5
R
ClaI (3259)
XhoI (3548)
ApaI (3557)
KpnI (3563)

FIG. 7

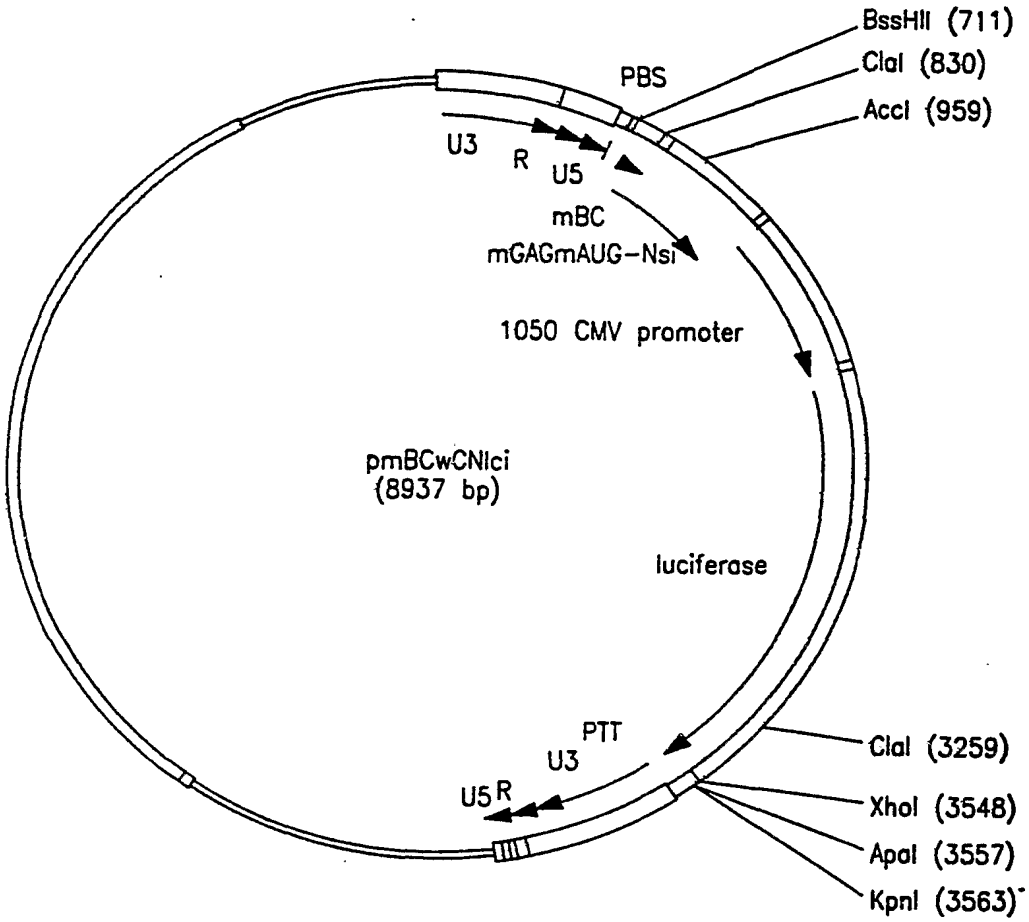

BssHII (711)
ClaI (830)
AccI (959)
PBS
U3
R
U5
mBC
mGAGmAUG-NsiI
1050 CMV promoter
pmBCwCNIci
(8937 bp)
luciferase
PTT
U3
U5
R
ClaI (3259)
XhoI (3548)
ApaI (3557)
KpnI (3563)

FIG. 8

```
   1  CCTGGCCATT GCATACGTTG TATCCATATC ATAATATGTA CATTTATATT GGCTCATGTC CAACATTACC
  71  GCCATGTTGA CATTGATTAT TGACTAGTTA TTAATAGTAA TCAATTACGG GGTCATTAGT TCATAGCCCA
 141  TATATGGAGT TCCGCGTTAC ATAACTTACG GTAAATGGCC CGCCTGGCTG ACCGCCCAAC GACCCCCGCC
 211  CATTGACGTC AATAATGACG TATGTTCCCA TAGTAACGCC AATAGGGACT TTCCATTGAC GTCAATGGGT
 281  GGAGTATTTA CGGTAAACTG CCCACTTGGC AGTACATCAA GTGTATCATA TGCCAAGTAC GCCCCCTATT
 351  GACGTCAATG ACGGTAAATG GCCCGCCTGG CATTATGCCC AGTACATGAC CTTATGGGAC TTTCCTACTT
 421  GGCAGTACAT CTACGTATTA GTCATCGCTA TTACCATGGT GATGCGGTTT TGGCAGTACA TCAATGGGCG
 491  TGGATAGCGG TTTGACTCAC GGGGATTTCC AAGTCTCCAC CCCATTGACG TCAATGGGAG TTTGTTTTGG
 561  CACCAAAATC AACGGGACTT TCCAAAATGT CGTAACAACT CCGCCCCATT GACGCAAATG GGCGGTAGGC
 631  GTGTACGGTG GGAGGTCTAT ATAAGCAGAG CTCGTTTAGT GAACCGTCAG ATCGCCTGGA GACGCCATCC
                                                                    SalI      (758)
 701  ACGCTGTTTT GACCTCCATA GAAGACACCG GGACCGATCC AGCCTCCGCG GGCGCGCGTC GACAGAGAGA
 771  TGGGTGCGAG AGCGTCAGTA TTAAGCGGGG GAGAATTAGA TCGATGGGAA AAAATTCGGT TAAGGCCAGG
 841  GGGAAAGAAG AAGTACAAGC TAAAGCACAT CGTATGGGCA AGCAGGGAGC TAGAACGATT CGCAGTTAAT
 911  CCTGGCCTGT TAGAAACATC AGAAGGCTGT AGACAAATAC TGGGACAGCT ACAACCATCC CTTCAGACAG
 981  GATCAGAGGA GCTTCGATCA CTATACAACA CAGTAGCAAC CCTCTATTGT GTGCACCAGC GGATCGAGAT
1051  CAAGGACACC AAGGAAGCTT TAGACAAGAT AGAGGAAGAG CAAAACAAGT CCAAGAAGAA GGCCCAGCAG
1121  GCAGCAGCTG ACACAGGACA CAGCAATCAG GTCAGCCAAA ATTACCCTAT AGTGCAGAAC ATCCAGGGGC
1191  AAATGGTACA TCAGGCCATA TCACCTAGAA CTTTAAATGC ATGGGTAAAA GTAGTAGAAG AGAAGGCTTT
1261  CAGCCCAGAA GTGATACCCA TGTTTTCAGC ATTATCAGAA GGAGCCACCC CACAGGACCT GAACACGATG
1331  TTGAACACCG TGGGGGGACA TCAAGCAGCC ATGCAAATGT TAAAAGAGAC CATCAATGAG GAAGCTGCAG
1401  AATGGGATAG AGTGCATCCA GTGCATGCAG GGCCTATTGC ACCAGGCCAG ATGAGAGAAC CAAGGGGAAG
1471  TGACATAGCA GGAACTACTA GTACCCTTCA GGAACAAATA GGATGGATGA CAAATAATCC ACCTATCCCA
1541  GTAGGAGAGA TCTACAAGAG GTGGATAATC CTGGGATTGA ACAAGATCGT GAGGATGTAT AGCCCTACCA
1611  GCATTCTGGA CATAAGACAA GGACCAAAGG AACCCTTTAG AGACTATGTA GACCGGTTCT ATAAAACTCT
1681  AAGAGCTGAG CAAGCTTCAC AGGAGGTAAA AAATTGGATG ACAGAAACCT TGTTGGTCCA AAATGCGAAC
1751  CCAGATTGTA AGACCATCCT GAAGGCTCTC GGCCCAGCGG CTACACTAGA AGAAATGATG ACAGCATGTC
1821  AGGGAGTAGG AGGACCCGGC CATAAGGCAA GAGTTTTGGC CGAGGCGATG AGCCAGGTGA CGAACTCGGC
```

FIG. 9

1891 GACCATAATG ATGCAGAGAG GCAACTTCCG GAACCAGCGG AAGATCGTCA AGTGCTTCAA TTGTGGCAAA

1961 GAAGGGCACA CCGCCAGGAA CTGCCGGGCC CCCCGGAAGA AGGGCTGTTG GAAATGTGGA AAGGAAGGAC

2031 ACCAAATGAA AGATTGTACT GAGAGACAGG CTAATTTTTT AGGGAAGATC TGGCCTTCCT ACAAGGGAAG

2101 GCCAGGGAAT TTTCTTCAGA GCAGACCAGA GCCAACAGCC CCACCAGAAG AGAGCTTCAG GTCTGGGGTA

2171 GAGACAACAA CTCCCCCTCA GAAGCAGGAG CCGATAGACA AGGAACTGTA TCCTTTAACT TCCCTCAGAT

2241 CACTCTTTGG CAACGACCCC TCGTCACAGT AAGGATCGGG GGGCAACTCA AGGAAGCGCT GCTCGATACA

2311 GGAGCAGATG ATACAGTATT AGAAGAAATG AGTTTGCCAG GAAGATGGAA ACCAAAAAATG ATAGGGGGGA

2381 TCGGGGGCTT CATCAAGGTG AGGCAGTACG ACCAGATACT CATAGAAATC TGTGGACATA AAGCTATAGG

2451 TACAGTATTA GTAGGACCTA CACCTGTCAA CATAATTGGA AGAAATCTGT TGACCCAGAT CGGCTGCACC

2521 TTGAACTTCC CCATCAGCCC TATTGAGACG GTGCCCGTGA AGTTGAAGCC GGGGATGGAC GGCCCCAAGG

2591 TCAAGCAATG GCCATTGACG AAAGAGAAGA TCAAGGCCTT AGTCGAAATC TGTACAGAGA TGGAGAAGGA

2661 AGGGAAGATC AGCAAGATCG GGCCTGAGAA CCCCTACAAC ACTCCAGTCT TCGCAATCAA GAAGAAGGAC

2731 AGTACCAAGT GGAGAAAGCT GGTGGACTTC AGAGAGCTGA ACAAGAGAAC TCAGGACTTC TGGGAAGTTC

2801 AGCTGGGCAT CCCACATCCC GCTGGGTTGA AGAAGAAGAA GTCAGTGACA GTGCTGGATG TGGGTGATGC

2871 CTACTTCTCC GTTCCCTTGG ACGAGGACTT CAGGAAGTAC ACTGCCTTCA CGATACCTAG CATCAACAAC

2941 GAGACACCAG GCATCCGCTA CCAGTACAAC GTGCTGCCAC AGGGATGGAA GGGATCACCA GCCATCTTTC

3011 AAAGCAGCAT GACCAAGATC CTGGAGCCCT TCCGCAAGCA AAACCCAGAC ATCGTGATCT ATCAGTACAT

3081 GGACGACCTC TACGTAGGAA GTGACCTGGA GATCGGGCAG CACAGGACCA AGATCGAGGA GCTGAGACAG

3151 CATCTGTTGA GGTGGGGACT GACCACACCA GACAAGAAGC ACCAGAAGGA ACCTCCCTTC CTGTGGATGG

3221 GCTACGAACT GCATCCTGAC AAGTGGACAG TGCAGCCCAT CGTGCTGCCT GAGAAGGACA GCTGGACTGT

3291 GAACGACATA CAGAAGCTCG TGGGCAAGTT GAACTGGGCA AGCCAGATCT ACCCAGGCAT CAAAGTTAGG

3361 CAGCTGTGCA AGCTGCTTCG AGGAACCAAG GCACTGACAG AAGTGATCCC ACTGACAGAG GAAGCAGAGC

3431 TAGAACTGGC AGAGAACCGA GAGATCCTGA AGGAGCCAGT ACATGGAGTG TACTACGACC CAAGCAAGGA

3501 CCTGATCGCA GAGATCCAGA AGCAGGGGCA AGGCCAATGG ACCTACCAAA TCTACCAGGA GCCCTTCAAG

3571 AACCTGAAGA CAGGCAAGTA CGCAAGGATG AGGGGTGCCC ACACCAACGA TGTGAAGCAG CTGACAGAGG

3641 CAGTGCAGAA GATCACCACA GAGAGCATCG TGATCTGGGG CAAGACTCCC AAGTTCAAGC TGCCCATACA

3711 GAAGGAGACA TGGGAGACAT GGTGGACCGA GTACTGGCAA GCCACCTGGA TCCCTGAGTG GGAGTTCGTG

```
3781  AACACCCCTC CCTTGGTGAA ACTGTGGTAT CAGCTGGAGA AGGAACCCAT CGTGGGAGCA GAGACCTTCT
3851  ACGTGGATGG GGCAGCCAAC AGGGAGACCA AGCTGGGCAA GGCAGGCTAC GTGACCAACC GAGGACGACA
3921  GAAAGTGGTG ACCCTGACTG ACACCACCAA CCAGAAGACT GAGCTGCAAG CCATCTACCT AGCTCTGCAA
3991  GACAGCGGAC TGGAAGTGAA CATCGTGACA GACTCACAGT ACGCACTGGG CATCATCCAA GCACAACCAG
4061  ACCAATCCGA GTCAGAGCTG GTGAACCAGA TCATCGAGCA GCTGATCAAG AAGGAGAAAG TGTACCTGGC
4131  ATGGGTACCA GCACACAAAG GAATTGGAGG AAATGAACAA GTAGATAAAT TAGTCAGTGC TGGGATCCGG
4201  AAGGTGCTGT TCCTGGACGG GATCGATAAG GCCCAAGATG AACATGAGAA GTACCACTCC AACTGGCGCG
4271  CTATGGCCAG CGACTTCAAC CTGCCACCTG TAGTAGCAAA AGAAATAGTA GCCAGCTGTG ATAAATGTCA
4341  GCTAAAAGGA GAAGCCATGC ATGGACAAGT AGACTGTAGT CCAGGAATAT GGCAGCTGGA CTGCACGCAC
4411  CTGGAGGGGA AGGTGATCCT GGTAGCAGTT CATGTAGCCA GTGGATATAT AGAAGCAGAA GTTATCCCTG
4481  CTGAAACTGG GCAGGAAACA GCATATTTTC TTTTAAAATT AGCAGGAAGA TGGCCAGTAA AAACAATACA
4551  CACGGACAAC GGAAGCAACT TCACTGGTGC TACGGTTAAG GCCGCCTGTT GGTGGGCGGG AATCAAGCAG
4621  GAATTTGGAA TTCCCTACAA TCCCCAATCG CAAGGAGTCG TGGAGAGCAT GAACAAGGAG CTGAAGAAGA
4691  TCATCGGACA AGTGAGGGAT CAGGCTGAGC ACCTGAAGAC AGCAGTGCAG ATGGCAGTGT TCATCCACAA
4761  CTTCAAAAGA AAAGGGGGGA TTGGGGGGTA CAGTGCAGGG GAAAGGATCG TGGACATCAT CGCCACCGAC
4831  ATCCAAACCA AGGAGCTGCA GAAGCAGATC ACCAAGATCC AGAACTTCCG GGTGTACTAC CGCGACAGCC
4901  GCAACCCACT GTGGAAGGGA CCAGCAAAGC TCCTCTGGAA GGGAGAGGGG GCAGTGGTGA TCCAGGACAA
4971  CAGTGACATC AAAGTGGTGC CAAGGCGCAA GGCCAAGATC ATCCGCGACT ATGGAAAACA GATGGCAGGT
5041  GATGATTGTG TGGCAAGTAG ACAGGATGAG GATTAGAACC TGGAAGAGCC TGGTGAAGCA CCATATGGCG
                                  NheI  (5117)
            BstBI   (5111)
5111  TTCGAAGCTA GCCTCGAGAT CCAGATCTGC TGTGCCTTCT AGTTGCCAGC CATCTGTTGT TTGCCCCTCC
5181  CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG TCCTTTCCTA ATAAAATGAG GAAATTGCAT
5251  CGCATTGTCT GAGTAGGTGT CATTCTATTC TGGGGGGTGG GGTGGGGCAG CACAGCAAGG GGGAGGATTG
5321  GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT ATGCGTACCC AGGTGCTGAA GAATTGACCC
5391  GGTTCCTCCT GGGCCAGAAA GAAGCAGGCA CATCCCCTTC TCTCTGACAC ACCCTGTCCA CGCCCCTGGT
5461  TCTTAGTTCC AGCCCCACTC ATAGGACACT CATAGCTCAG GAGGGCTCCG CCTTCAATCC CACCCGCTAA
5531  AGTACTTGGA GCGGTCTCTC CCTCCCTCAT CAGCCCACCA AACCAAACCT AGCCTCCAAG AGTGGGAAGA
```

```
5601  AATTAAAGCA AGATAGGCTA TTAAGTGCAG AGGGAGAGAA AATCCCTCCA ACATGTGAGG AAGTAATGAG

5671  AGAAATCATA GAATTTCTTC CGCTTCCTCG CTCACTGACT CGCTGCGCTC GGTCGTTCGG CTGCGGCGAG

5741  CGGTATCAGC TCACTCAAAG GCGGTAATAC GGTTATCCAC AGAATCAGGG GATAACGCAG GAAAGAACAT
5811  GTGAGCAAAA GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG GCCGCGTTGC TGGCGTTTTT CCATAGGCTC
5881  CGCCCCCCTG ACGAGCATCA CAAAAATCGA CGCTCAAGTC AGAGGTGGCG AAACCCGACA GGACTATAAA
5951  GATACCAGGC GTTTCCCCCT GGAAGCTCCC TCGTGCGCTC TCCTGTTCCG ACCCTGCCGC TTACCGGATA
6021  CCTGTCCGCC TTTCTCCCTT CGGGAAGCGT GGCGCTTTCT CAATGCTCAC GCTGTAGGTA TCTCAGTTCG
6091  GTGTAGGTCG TTCGCTCCAA GCTGGGCTGT GTGCACGAAC CCCCCGTTCA GCCCGACCGC TGCGCCTTAT
6161  CCGGTAACTA TCGTCTTGAG TCCAACCCGG TAAGACACGA CTTATCGCCA CTGGCAGCAG CCACTGGTAA
6231  CAGGATTAGC AGAGCGAGGT ATGTAGGCGG TGCTACAGAG TTCTTGAAGT GGTGGCCTAA CTACGGCTAC
6301  ACTAGAAGGA CAGTATTTGG TATCTGCGCT CTGCTGAAGC CAGTTACCTT CGGAAAAAGA GTTGGTAGCT
6371  CTTGATCCGG CAAACAAACC ACCGCTGGTA GCGGTGGTTT TTTTGTTTGC AAGCAGCAGA TTACGCGCAG
6441  AAAAAAAGGA TCTCAAGAAG ATCCTTTGAT CTTTTCTACG GGGTCTGACG CTCAGTGGAA CGAAAACTCA
6511  CGTTAAGGGA TTTTGGTCAT GAGATTATCA AAAAGGATCT TCACCTAGAT CCTTTTAAAT TAAAAATGAA
6581  GTTTTAAATC AATCTAAAGT ATATATGAGT AAACTTGGTC TGACAGTTAC CAATGCTTAA TCAGTGAGGC
6651  ACCTATCTCA GCGATCTGTC TATTTCGTTC ATCCATAGTT GCCTGACTCC GGGGGGGGGG GGCGCTGAGG
6721  TCTGCCTCGT GAAGAAGGTG TTGCTGACTC ATACCAGGCC TGAATCGCCC CATCATCCAG CCAGAAAGTG
6791  AGGGAGCCAC GGTTGATGAG AGCTTTGTTG TAGGTGGACC AGTTGGTGAT TTTGAACTTT TGCTTTGCCA
6861  CGGAACGGTC TGCGTTGTCG GGAAGATGCG TGATCTGATC CTTCAACTCA GCAAAAGTTC GATTTATTCA
6931  ACAAAGCCGC CGTCCCGTCA AGTCAGCGTA ATGCTCTGCC AGTGTTACAA CCAATTAACC AATTCTGATT
7001  AGAAAAACTC ATCGAGCATC AAATGAAACT GCAATTTATT CATATCAGGA TTATCAATAC CATATTTTTG
 271  PhePheGlu AspLeuMetL euHisPheGl nLeuLysAsn MetAspProA snAspIleGl yTyrLysGln
7071  AAAAAGCCGT TTCTGTAATG AAGGAGAAAA CTCACCGAGG CAGTTCCATA GGATGGCAAG ATCCTGGTAT
 248  PheLeuArgL ysGlnLeuSe rProSerPhe GluGlyLeuC ysAsnTrpLe uIleAlaLeu AspGlnTyrA
7141  CGGTCTGCGA TTCCGACTCG TCCAACATCA ATACAACCTA TTAATTTCCC CTCGTCAAAA ATAAGGTTAT
 224  rgAspAlaIl eGlyValArg GlyValAspI leCysGlyIl eLeuLysGly GluAspPheI leLeuAsnAs
7211  CAAGTGAGAA ATCACCATGA GTGACGACTG AATCCGGTGA GAATGGCAAA AGCTTATGCA TTTCTTTCCA
 201  pLeuSerPhe AspGlyHisT hrValValSe rAspProSer PheProLeuL euLysHisMe tGluLysTrp
7281  GACTTGTTCA ACAGGCCAGC CATTACGCTC GTCATCAAAA TCACTCGCAT CAACCAAACC GTTATTCATT
 178  ValGlnGluV alProTrpGl yAsnArgGlu AspAspPheA spSerAlaAs pValLeuGly AsnAsnMetA
7351  CGTGATTGCG CCTGAGCGAG ACGAAATACG CGATCGCTGT TAAAAGGACA ATTACAAACA GGAATCGAAT
 154  rgSerGlnAl aGlnAlaLeu ArgPheValA rgAspSerAs nPheProCys AsnCysValP roIleSerHi
7421  GCAACCGGCG CAGGAACACT GCCAGCGCAT CAACAATATT TTCACCTGAA TCAGGATATT CTTCTAATAC
 131  sLeuArgArg LeuPheValA laLeuAlaAs pValIleAsn GluGlySerA spProTyrGl uGluLeuVal
7491  CTGGAATGCT GTTTTCCCGG GGATCGCAGT GGTGAGTAAC CATGCATCAT CAGGAGTACG GATAAAATGC
 108  GlnPheAlaT hrLysGlyPr oIleAlaThr ThrLeuLeuT rpAlaAspAs pProThrArg IlePheHisL
7561  TTGATGGTCG GAAGAGGCAT AAATTCCGTC AGCCAGTTTA GTCTGACCAT CTCATCTGTA ACATCATTGG
  84  ysIleThrPr oLeuProMet PheGluThrL euTrpAsnLe uArgValMet GluAspThrV alAspAsnAl
7631  CAACGCTACC TTTGCCATGT TTCAGAAACA ACTCTGGCGC ATCGGGCTTC CCATACAATC GATAGATTGT
   61  aValSerGly LysGlyHisL ysLeuPheLe uGluProAla AspProLysG lyTyrLeuAr gTyrIleThr
7701  CGCACCTGAT TGCCCGACAT TATCGCGAGC CCATTTATAC CCATATAAAT CAGCATCCAT GTTGGAATTT
   38  AlaGlySerG lnGlyValAs nAspArgAla TrpLysTyrG lyTyrLeuAs pAlaAspMet AsnSerAsnL
7771  AATCGCGGCC TCGAGCAAGA CGTTTCCCGT TGAATATGGC TCATAACACC CCTTGTATTA CTGTTTATGT
   14  euArgProAr gSerCysSer ThrGluArgG lnIleHisSe rMet
7841  AAGCAGACAG TTTTATTGTT CATGATGATA TATTTTTATC TTGTGCAATG TAACATCAGA GATTTTGAGA
7911  CACAACGTGG CTTTCCCCCC CCCCCCATTA TTGAAGCATT TATCAGGGTT ATTGTCTCAT GAGCGGATAC
7981  ATATTTGAAT GTATTTAGAA AAATAAACAA ATAGGGGTTC CGCGCACATT TCCCCGAAAA GTGCCACCTG
8051  ACGTCTAAGA AACCATTATT ATCATGACAT TAACCTATAA AAATAGGCGT ATCACGAGGC CCTTTCGTCT
8121  CGCGCGTTTC GGTGATGACG GTGAAAACCT CTGACACATG CAGCTCCCGG AGACGGTCAC AGCTTGTCTG
8191  TAAGCGGATG CCGGGAGCAG ACAAGCCCGT CAGGGCGCGT CAGCGGGTGT TGGCGGGTGT CGGGGCTGGC
8261  TTAACTATGC GGCATCAGAG CAGATTGTAC GTAGAGTGCA CCATATGCGG TGTGAAATAC CGCACAGATG
8331  CGTAAGGAGA AAATACCGCA TCAGATTGGC TATTGG
```

```
   1  TGGAAGGGCT AATTTGGTCC CAAAAAAGAC AAGAGATCCT TGATCTGTGG ATCTACCACA CACAAGGCTA
  71  CTTCCCTGAT TGGCAGAACT ACACACCAGG GCCAGGGATC AGATATCCAC TGACCTTTGG ATGGTGCTTC
 141  AAGTTAGTAC CAGTTGAACC AGAGCAAGTA GAAGAGGCCA AATAAGGAGA GAAGAACAGC TTGTTACACC
 211  CTATGAGCCA GCATGGGATG GAGGACCCGG AGGGAGAAGT ATTAGTGTGG AAGTTTGACA GCCTCCTAGC
 281  ATTTCGTCAC ATGGCCCGAG AGCTGCATCC GGAGTACTAC AAAGACTGCT GACATCGAGC TTTCTACAAG
 351  GGACTTTCCG CTGGGGACTT TCCAGGGAGG TGTGGCCTGG GCGGGACTGG GGAGTGGCGA GCCCTCAGAT
 421  GCTACATATA AGCAGCTGCT TTTTGCCTGT ACTGGGTCTC TCTGGTTAGA CCAGATCTGA GCCTGGGAGC
 491  TCTCTGGCTA ACTAGGGAAC CCACTGCTTA AGCCTCAATA AAGCTTGCCT TGAGTGCTCA AAGTAGTGTG
 561  TGCCCGTCTG TTGTGTGACT CTGGTAACTA GAGATCCCTC AGACCCTTTT AGTCAGTGTG GAAAATCTCT
 631  AGCAGTGGCG CCCGAACAGG GACTTGAAAG CGAAAGTAAA GCCAGAGGAG ATCTCTCGAC GCAGGACTCG
                BssHII  (711)
 701  GCTTGCTGAA GCGCGCacgg caagaggcga ggggcggcgC ctgACgagGa cgccaaaaat tttgactagc
                                                                      ClaI  (830)
 771  ggaggctaga aggagagagC TCGGTGCGAG AGCGTCAGTA TCAAGCGGGG GAGAATTAGA TCGATGGGAA
 841  AAAATTCGGT TAAGGCCAGG GGGAAAGAAA AAATATAAAT TAAAACATAT AGTATGGGCA AGCAGGGAGC
                                                          AccI  (959)
 911  TAGAACGATT CGCAGTTAAT CCTGGCCTGT TAGAAACATC AGAAGGCTGT AGACAAATAC TGGGACAGCT
 981  ACAACCATCC CTTCAGACAG GATCAGAAGA ACTTAGATCA TTATATAATA CAGTAGCAAC CCTCTATTGT
1051  GTGCATCAAA GGATAGAGAT AAAAGACACC AAGGAAGCTT TAGACAAGAT AGAGGAAGAG CAAAACAAAA
```

FIG. 10

1121 GTAAGAAAAA AGCACAGCAA GCAGCAGCTG ACACAGGACA CAGCAATCAG GTCAGCCAAA ATTACCCTAT

1191 AGTGCAGAAC ATCCAGGGGC AAATGGTACA TCAGGCCATA TCACCTAGAA CTTTAAACGA TAAGCTTGGG

1261 AGTTCCGCGT TACATAACTT ACGGTAAATG GCCCGCCTGG CTGACCGCCC AACGACCCCC GCCCATTGAC

1331 GTCAATAATG ACGTATGTTC CCATAGTAAC GCCAATAGGG ACTTTCCATT GACGTCAATG GGTGGAGTAT

1401 TTACGGTAAA CTGCCCACTT GGCAGTACAT CAAGTGTATC ATATGCCAAG TACGCCCCCT ATTGACGTCA

1471 ATGACGGTAA ATGGCCCGCC TGGCATTATG CCCAGTACAT GACCTTATGG GACTTTCCTA CTTGGCAGTA

1541 CATCTACGTA TTAGTCATCG CTATTACCAT GGTGATGCGG TTTTGGCAGT ACATCAATGG GCGTGGATAG

1611 CGGTTTGACT CACGGGGATT TCCAAGTCTC CACCCCATTG ACGTCAATGG GAGTTTGTTT TGGCACCAAA

1681 ATCAACGGGA CTTTCCAAAA TGTCGTAACA ACTCCGCCCC ATTGACGCAA ATGGGCGGTA GGCGTGTACG

1751 GTGGGAGGTC TATATAAGCA GAGCTCGTTT AGTGAACCGT CAGATCGCCT GGAGACGCCA TCCACGCTGT

1821 TTTGACCTCC ATAGAAGACA CCGACTCTAG AGgatccATC TAAGTAAGCT TGGCATTCCG GTACTGTTGG

1891 TAAAATGGAA GACGCCAAAA ACATAAAGAA AGGCCCGGCG CCATTCTATC CTCTAGAGGA TGGAACCGCT

1961 GGAGAGCAAC TGCATAAGGC TATGAAGAGA TACGCCCTGG TTCCTGGAAC AATTGCTTTT ACAGATGCAC

2031 ATATCGAGGT GAACATCACG TACGCGGAAT ACTTCGAAAT GTCCGTTCGG TTGGCAGAAG CTATGAAACG

2101 ATATGGGCTG AATACAAATC ACAGAATCGT CGTATGCAGT GAAAACTCTC TTCAATTCTT TATGCCGGTG

2171 TTGGGCCCGT TATTTATCGG AGTTGCAGTT GCGCCCGCGA ACGACATTTA TAATGAACGT GAATTGCTCA

2241 ACAGTATGAA CATTTCGCAG CCTACCGTAG TGTTTGTTTC CAAAAAGGGG TTGCAAAAAA TTTTGAACGT

2311 GCAAAAAAAA TTACCAATAA TCCAGAAAAT TATTATCATG GATTCTAAAA CGGATTACCA GGGATTTCAG

2381 TCGATGTACA CGTTCGTCAC ATCTCATCTA CCTCCCGGTT TTAATGAATA CGATTTTGTA CCAGAGTCCT

2451 TTGATCGTGA CAAAACAATT GCACTGATAA TGAATTCCTC TGGATCTACT GGGTTACCTA AGGGTGTGGC

2521 CCTTCCGCAT AGAACTGCCT GCGTCAGATT CTCGCATGCC AGAGATCCTA TTTTTGGCAA TCAAATCATT

2591 CCGGATACTG CGATTTTAAG TGTTGTTCCA TTCCATCACG GTTTTGGAAT GTTTACTACA CTCGGATATT

2661 TGATATGTGG ATTTCGAGTC GTCTTAATGT ATAGATTTGA AGAAGAGCTG TTTTTACGAT CCCTTCAGGA

2731 TTACAAAATT CAAAGTGCGT TGCTAGTACC AACCCTATTT TCATTCTTCG CCAAAAGCAC TCTGATTGAC

2801 AAATACGATT TATCTAATTT ACACGAAATT GCTTCTGGGG GCGCACCTCT TTCGAAAGAA GTCGGGGAAG

2871 CGGTTGCAAA ACGCTTCCAT CTTCCAGGGA TACGACAAGG ATATGGGCTC ACTGAGACTA CATCAGCTAT

2941 TCTGATTACA CCCGAGGGGG ATGATAAACC GGGCGCGGTC GGTAAAGTTG TTCCATTTTT TGAAGCGAAG

3011 GTTGTGGATC TGGATACCGG GAAAACGCTG GGCGTTAATC AGAGAGGCGA ATTATGTGTC AGAGGACCTA

3081 TGATTATGTC CGGTTATGTA AACAATCCGG AAGCGACCAA CGCCTTGATT GACAAGGATG GATGGCTACA

3151 TTCTGGAGAC ATAGCTTACT GGGACGAAGA CGAACACTTC TTCATAGTTG ACCGCTTGAA GTCTTTAATT

ClaI (3259)

3221 AAATACAAAG GATATCAGGT GGCCCCCGCT GAATTGGAAT CGATATTGTT ACAACACCCC AACATCTTCG

3291 ACGCGGGCGT GGCAGGTCTT CCCGACGATG ACGCCGGTGA ACTTCCCGCC GCCGTTGTTG TTTTGGAGCA

3361 CGGAAAGACG ATGACGGAAA AAGAGATCGT GGATTACGTC GCCAGTCAAG TAACAACCGC GAAAAAGTTG

3431 CGCGGAGGAG TTGTGTTTGT GGACGAAGTA CCGAAAGGTC TTACCGGAAA ACTCGACGCA AGAAAAATCA

ApaI (3557)

XhoI (3548) KpnI(3563

3501 GAGAGATCCT CATAAAGGCC AAGAAGGGCG GAAAGTCCAA ATTGTAAcTC GAGGGGGGGC CCGGTACCTT

```
3571  TAAGACCAAT GACTTACAAG GCAGCTGTAG ATCTTAGCCA CTTTTTAAAA GAAAAGGGGG GACTGGAAGG

3641  GCTAATTCAC TCCCAAAGAA GACAAGATAT CCTTGATCTG TGGATCTACC ACACACAAGG CTACTTCCCT

3711  GATTGGCAGA ACTACACACC AGGGCCAGGG GTCAGATATC CACTGACCTT TGGATGGTGC TACAAGCTAG

3781  TACCAGTTGA GCCAGATAAG GTAGAAGAGG CCAATAAAGG AGAGAACACC AGCTTGTTAC ACCCTGTGAG

3851  CCTGCATGGA ATGGATGACC CTGAGAGAGA AGTGTTAGAG TGGAGGTTTG ACAGCCGCCT AGCATTTCAT

3921  CACGTGGCCC GAGAGCTGCA TCCGGAGTAC TTCAAGAACT GCTGACATCG AGCTTGCTAC AAGGGACTTT

3991  CCGCTGGGGA CTTTCCAGGG AGGCGTGGCC TGGGCGGGAC TGGGGAGTGG CGAGCCCTCA GATGCTGCAT

4061  ATAAGCAGCT GCTTTTTGCC TGTACTGGGT CTCTCTGGTT AGACCAGATC TGAGCCTGGG AGCTCTCTGG

4131  CTAACTAGGG AACCCACTGC TTAAGCCTCA ATAAAGCTTG CCTTGAGTGC TTCAAGTAGT GTGTGCCCGT

4201  CTGTTGTGTG ACTCTGGTAA CTAGAGATCC CTCAGACCCT TTTAGTCAGT GTGGAAAATC TCTAGCACCC

4271  CCCAGGAGGT AGAGGTTGCA GTGAGCCAAG ATCGCGCCAC TGCATTCCAG CCTGGGCAAG AAAACAAGAC
4341  TGTCTAAAAT AATAATAATA AGTTAAGGGT ATTAAATATA TTTATACATG GAGGTCATAA AAATATATAT
4411  ATTTGGGCTG GGCGCAGTGG CTCACACCTG CGCCCGGCCC TTTGGGAGGC CGAGGCAGGT GGATCACCTG
4481  AGTTTGGGAG TTCCAGACCA GCCTGACCAA CATGGAGAAA CCCCTTCTCT GTGTATTTTT ATGAGATTTT
4551  ATTTTATGTG TATTTTATTC ACAGGTATTT CTGGAAAACT GAAACTGTTT TTCCTCTACT CTGATACCAC
4621  AAGAATCATC AGCACAGAGG AAGACTTCTG TGATCAAATG TGGTGGGAGA GGGAGGTTTT CACCAGCACA
4691  TGAGCAGTCA GTTCTGCCGC AGACTCGGCG GGTGTCCTTC GGTTCAGTTC CAACACCGCC TGCCTGGAGA
4761  GAGGTCAGAC CACAGGGTGA GGGCTCAGTC CCCAAGACAT AAACACCCAA GACATAAACA CCCAACAGGT
4831  CCACCCCGCC TGCTGCCCAG GCAGAGCCGA TTCACCAAGA CGGGAATTAG GATAGAGAAA GAGTAAGTCA
4901  CACAGAGCCG GCTGTGCGGG AGAACGGAGT TCTATTATGA CTCAAATCAG TCTCCCCAAG CATTCGGGGA
4971  TCAGAGTTTT TAAGGATAAC TTAGTGTGTA GGGGGCCAGT GAGTTGGAGA TGAAAGCGTA GGGAGTCGAA
5041  GGTGTCCTTT TGCGCCGAGT CAGTTCCTGG GTGGGGGCCA CAAGATCGGA TGAGCCAGTT TATCAATCCG
5111  GGGGTGCCAG CTGATCCATG GAGTGCAGGG TCTGCAAAAT ATCTCAAGCA CTGATTGATC TTAGGTTTTA
5181  CAATAGTGAT GTTACCCCAG GAACAATTTG GGGAAGGTCA GAATCTTGTA GCCTGTAGCT GCATGACTCC
5251  TAAACCATAA TTTCTTTTTT GTTTTTTTTT TTTTATTTTT GAGACAGGGT CTCACTCTGT CACCTAGGCT
5321  GGAGTGCAGT GGTGCAATCA CAGCTCACTG CAGCCCCTAG AGCGGCCGCC ACCGCGGTGG AGCTCCAATT
5391  CGCCCTATAG TGAGTCGTAT TACAATTCAC TGGCCGTCGT TTTACAACGT CGTGACTGGG AAAACCCTGG
5461  CGTTACCCAA CTTAATCGCC TTGCAGCACA TCCCCCTTTC GCCAGCTGGC GTAATAGCGA AGAGGCCCGC
5531  ACCGATCGCC CTTCCCAACA GTTGCGCAGC CTGAATGGCG AATGGCGCGA AATTGTAAAC GTTAATATTT
5601  TGTTAAAATT CGCGTTAAAT TTTTGTTAAA TCAGCTCATT TTTTAACCAA TAGGCCGAAA TCGGCAAAAT
5671  CCCTTATAAA TCAAAAGAAT AGACCGAGAT AGGGTTGAGT GTTGTTCCAG TTTGGAACAA GAGTCCACTA
5741  TTAAAGAACG TGGACTCCAA CGTCAAAGGG CGAAAAACCG TCTATCAGGG CGATGGCCCA CTACGTGAAC
5811  CATCACCCTA ATCAAGTTTT TTGGGGTCGA GGTGCCGTAA AGCACTAAAT CGGAACCCTA AAGGGAGCCC
5881  CCGATTTAGA GCTTGACGGG GAAAGCCGGC GAACGTGGCG AGAAAGGAAG GGAAGAAAGC GAAAGGAGCG
```

```
5951  GGCGCTAGGG CGCTGGCAAG TGTAGCGGTC ACGCTGCGCG TAACCACCAC ACCCGCCGCG CTTAATGCGC
6021  CGCTACAGGG CGCGTCCCAG GTGGCACTTT TCGGGGAAAT GTGCGCGGAA CCCCTATTTG TTTATTTTTC
6091  TAAATACATT CAAATATGTA TCCGCTCATG AGACAATAAC CCTGATAAAT GCTTCAATAA TATTGAAAAA
6161  GGAAGAGTAT GAGTATTCAA CATTTCCGTG TCGCCCTTAT TCCCTTTTTT GCGGCATTTT GCCTTCCTGT
6231  TTTTGCTCAC CCAGAAACGC TGGTGAAAGT AAAAGATGCT GAAGATCAGT TGGGTGCACG AGTGGGTTAC
6301  ATCGAACTGG ATCTCAACAG CGGTAAGATC CTTGAGAGTT TTCGCCCCGA AGAACGTTTT CCAATGATGA
6371  GCACTTTTAA AGTTCTGCTA TGTGGCGCGG TATTATCCCG TATTGACGCC GGGCAAGAGC AACTCGGTCG
6441  CCGCATACAC TATTCTCAGA ATGACTTGGT TGAGTACTCA CCAGTCACAG AAAAGCATCT TACGGATGGC
6511  ATGACAGTAA GAGAATTATG CAGTGCTGCC ATAACCATGA GTGATAACAC TGCGGCCAAC TTACTTCTGA
6581  CAACGATCGG AGGACCGAAG GAGCTAACCG CTTTTTTGCA CAACATGGGG GATCATGTAA CTCGCCTTGA
6651  TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT ACCAAACGAC GAGCGTGACA CCACGATGCC TGTAGCAATG
6721  GCAACAACGT TGCGCAAACT ATTAACTGGC GAACTACTTA CTCTAGCTTC CCGGCAACAA TTAATAGACT
6791  GGATGGAGGC GGATAAAGTT GCAGGACCAC TTCTGCGCTC GGCCCTTCCG GCTGGCTGGT TTATTGCTGA
6861  TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CGGTATCATT GCAGCACTGG GGCCAGATGG TAAGCCCTCC
6931  CGTATCGTAG TTATCTACAC GACGGGGAGT CAGGCAACTA TGGATGAACG AAATAGACAG ATCGCTGAGA
7001  TAGGTGCCTC ACTGATTAAG CATTGGTAAC TGTCAGACCA AGTTTACTCA TATATACTTT AGATTGATTT
7071  AAAACTTCAT TTTTAATTTA AAAGGATCTA GGTGAAGATC CTTTTTGATA ACTTCATGAC CAAAATCCCT
7141  TAACGTGAGT TTTCGTTCCA CTGAGCGTCA GACCCCGTAG AAAAGATCAA AGGATCTTCT TGAGATCCTT
7211  TTTTTCTGCG CGTAATCTGC TGCTTGCAAA CAAAAAAACC ACCGCTACCA GCGGTGGTTT GTTTGCCGGA
7281  TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC AGCAGAGCGC AGATACCAAA TACTGTCCTT
7351  CTAGTGTAGC CGTAGTTAGG CCACCACTTC AAGAACTCTG TAGCACCGCC TACATACCTC GCTCTGCTAA
7421  TCCTGTTACC AGTGGCTGCT GCCAGTGGCG ATAAGTCGTG TCTTACCGGG TTGGACTCAA GACGATAGTT
7491  ACCGGATAAG GCGCAGCGGT CGGGCTGAAC GGGGGGTTCG TGCACACAGC CCAGCTTGGA GCGAACGACC
7561  TACACCGAAC TGAGATACCT ACAGCGTGAG CTATGAGAAA GCGCCACGCT TCCCGAAGGG AGAAAGGCGG
7631  ACAGGTATCC GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG CACGAGGGAG CTTCCAGGGG GAAACGCCTG
7701  GTATCTTTAT AGTCCTGTCG GGTTTCGCCA CCTCTGACTT GAGCGTCGAT TTTTGTGATG CTCGTCAGGG
7771  GGGCGGAGCC TATGGAAAAA CGCCAGCAAC GCGGCCTTTT TACGGTTCCT GGCCTTTTGC TGGCCTTTTG
7841  CTCACATGTT CTTTCCTGCG TTATCCCCTG ATTCTGTGGA TAACCGTATT ACCGCCTTTG AGTGAGCTGA
7911  TACCGCTCGC CGCAGCCGAA CGACCGAGCG CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA GCGCCCAATA
7981  CGCAAACCGC CTCTCCCCGC GCGTTGGCCG ATTCATTAAT GCAGCTGGCA CGACAGGTTT CCCGACTGGA
8051  AAGCGGGCAG TGAGCGCAAC GCAATTAATG TGAGTTAGCT CACTCATTAG GCACCCCAGG CTTTACACTT
8121  TATGCTTCCG GCTCGTATGT TGTGTGGAAT TGTGAGCGGA TAACAATTTC ACACAGGAAA CAGCTATGAC
8191  CATGATTACG CCAAGCTCGG AATTAACCCT CACTAAAGGG AACAAAAGCT GCTGCAGGGT CCCTAACTGC
8261  CAAGCCCCAC AGTGTGCCCT GAGGCTGCCC CTTCCTTCTA GCGGCTGCCC CCACTCGGCT TTGCTTTCCC
8331  TAGTTTCAGT TACTTGCGTT CAGCCAAGGT CTGAAACTAG GTGCGCACAG AGCGGTAAGA CTGCGAGAGA
8401  AAGAGACCAG CTTTACAGGG GGTTTATCAC AGTGCACCCT GACAGTCGTC AGCCTCACAG GGGGTTTATC
8471  ACATTGCACC CTGACAGTCG TCAGCCTCAC AGGGGGTTTA TCACAGTGCA CCCTTACAAT CATTCCATTT
8541  GATTCACAAT TTTTTTAGTC TCTACTGTGC CTAACTTGTA AGTTAAATTT GATCAGAGGT GTGTTCCCAG
8611  AGGGGAAAAC AGTATATACA GGGTTCAGTA CTATCGCATT TCAGGCCTCC ACCTGGGTCT TGGAATGTGT
8681  CCCCCGAGGG GTGATGACTA CCTCAGTTGG ATCTCCACAG GTCACAGTGA CACAAGATAA CCAAGACACC
8751  TCCCAAGGCT ACCACAATGG GCCGCCCTCC ACGTGCACAT GGCCGGAGGA ACTGCCATGT CGGAGGTGCA
8821  AGCACACCTG CGCATCAGAG TCCTTGGTGT GGAGGGAGGG ACCAGCGCAG CTTCCAGCCA TCCACCTGAT
8891  GAACAGAACC TAGGGAAAGC CCCAGTTCTA CTTACACCAG GAAAGGC
```

```
   1  TGGAAGGGCT AATTTGGTCC CAAAAAAGAC AAGAGATCCT TGATCTGTGG ATCTACCACA CACAAGGCTA

  71  CTTCCCTGAT TGGCAGAACT ACACACCAGG GCCAGGGATC AGATATCCAC TGACCTTTGG ATGGTGCTTC

 141  AAGTTAGTAC CAGTTGAACC AGAGCAAGTA GAAGAGGCCA AATAAGGAGA GAAGAACAGC TTGTTACACC

 211  CTATGAGCCA GCATGGGATG GAGGACCCGG AGGGAGAAGT ATTAGTGTGG AAGTTTGACA GCCTCCTAGC

 281  ATTTCGTCAC ATGGCCCGAG AGCTGCATCC GGAGTACTAC AAAGACTGCT GACATCGAGC TTTCTACAAG

 351  GGACTTTCCG CTGGGGACTT TCCAGGGAGG TGTGGCCTGG GCGGGACTGG GGAGTGGCGA GCCCTCAGAT

 421  GCTACATATA AGCAGCTGCT TTTTGCCTGT ACTGGGTCTC TCTGGTTAGA CCAGATCTGA GCCTGGGAGC

 491  TCTCTGGCTA ACTAGGGAAC CCACTGCTTA AGCCTCAATA AAGCTTGCCT TGAGTGCTCA AGTAGTGTG

 561  TGCCCGTCTG TTGTGTGACT CTGGTAACTA GAGATCCCTC AGACCCTTTT AGTCAGTGTG GAAAATCTCT

 631  AGCAGTGGCG CCCGAACAGG GACTTGAAAG CGAAAGTAAA GCCAGAGGAG ATCTCTCGAC GCAGGACTCG

                BssHII  (711)
 701  GCTTGCTGAA GCGCGCacgg caagaggcga ggggcggcgC ctgACgagGa cgccaaaaat tttgactagc

                                                                    ClaI  (830)
 771  ggaggctaga aggagagagC TCGGTGCGAG AGCGTCAGTA TTAAGCGGGG GAGAATTAGA TCGATGGGAA

 841  AAAATTCGGT TAAGGCCAGG GGGAAAGAAG AAGTACAAGC TAAAGCACAT CGTATGGGCA AGCAGGGAGC

                                                          AccI  (959)
 911  TAGAACGATT CGCAGTTAAT CCTGGCCTGT TAGAAACATC AGAAGGCTGT AGACAAATAC TGGGACAGCT

 981  ACAACCATCC CTTCAGACAG GATCAGAGGA GCTTCGATCA CTATACAACA CAGTAGCAAC CCTCTATTGT

1051  GTGCACCAGC GGATCGAGAT CAAGGACACC AAGGAAGCTT TAGACAAGAT AGAGGAAGAG CAAAACAAGT

1121  CCAAGAAGAA GGCCCAGCAG GCAGCAGCTG ACACAGGACA CAGCAATCAG GTCAGCCAAA ATTACCCTAT
```

FIG. 11

1191 AGTGCAGAAC ATCCAGGGGC AAATGGTACA TCAGGCCATA TCACCTAGAA CTTTAAACGA TAAGCTTGGG

1261 AGTTCCGCGT TACATAACTT ACGGTAAATG GCCCGCCTGG CTGACCGCCC AACGACCCCC GCCCATTGAC

1331 GTCAATAATG ACGTATGTTC CCATAGTAAC GCCAATAGGG ACTTTCCATT GACGTCAATG GGTGGAGTAT

1401 TTACGGTAAA CTGCCCACTT GGCAGTACAT CAAGTGTATC ATATGCCAAG TACGCCCCCT ATTGACGTCA

1471 ATGACGGTAA ATGGCCCGCC TGGCATTATG CCCAGTACAT GACCTTATGG GACTTTCCTA CTTGGCAGTA

1541 CATCTACGTA TTAGTCATCG CTATTACCAT GGTGATGCGG TTTTGGCAGT ACATCAATGG GCGTGGATAG

1611 CGGTTTGACT CACGGGGATT TCCAAGTCTC CACCCCATTG ACGTCAATGG GAGTTTGTTT TGGCACCAAA

1681 ATCAACGGGA CTTTCCAAAA TGTCGTAACA ACTCCGCCCC ATTGACGCAA ATGGGCGGTA GGCGTGTACG

1751 GTGGGAGGTC TATATAAGCA GAGCTCGTTT AGTGAACCGT CAGATCGCCT GGAGACGCCA TCCACGCTGT

1821 TTTGACCTCC ATAGAAGACA CCGACTCTAG AGgatccATC TAAGTAAGCT TGGCATTCCG GTACTGTTGG

1891 TAAAATGGAA GACGCCAAAA ACATAAAGAA AGGCCCGGCG CCATTCTATC CTCTAGAGGA TGGAACCGCT

1961 GGAGAGCAAC TGCATAAGGC TATGAAGAGA TACGCCCTGG TTCCTGGAAC AATTGCTTTT ACAGATGCAC

2031 ATATCGAGGT GAACATCACG TACGCGGAAT ACTTCGAAAT GTCCGTTCGG TTGGCAGAAG CTATGAAACG

2101 ATATGGGCTG AATACAAATC ACAGAATCGT CGTATGCAGT GAAAACTCTC TTCAATTCTT TATGCCGGTG

2171 TTGGGCGCGT TATTTATCGG AGTTGCAGTT GCGCCCGCGA ACGACATTTA TAATGAACGT GAATTGCTCA

2241 ACAGTATGAA CATTTCGCAG CCTACCGTAG TGTTTGTTTC CAAAAAGGGG TTGCAAAAAA TTTTGAACGT

2311 GCAAAAAAAA TTACCAATAA TCCAGAAAAT TATTATCATG GATTCTAAAA CGGATTACCA GGGATTTCAG

2381 TCGATGTACA CGTTCGTCAC ATCTCATCTA CCTCCCGGTT TTAATGAATA CGATTTTGTA CCAGAGTCCT

2451 TTGATCGTGA CAAAACAATT GCACTGATAA TGAATTCCTC TGGATCTACT GGGTTACCTA AGGGTGTGGC

2521 CCTTCCGCAT AGAACTGCCT GCGTCAGATT CTCGCATGCC AGAGATCCTA TTTTTGGCAA TCAAATCATT

2591 CCGGATACTG CGATTTTAAG TGTTGTTCCA TTCCATCACG GTTTTGGAAT GTTTACTACA CTCGGATATT

2661 TGATATGTGG ATTTCGAGTC GTCTTAATGT ATAGATTTGA AGAAGAGCTG TTTTTACGAT CCCTTCAGGA

2731 TTACAAAATT CAAAGTGCGT TGCTAGTACC AACCCTATTT TCATTCTTCG CCAAAAGCAC TCTGATTGAC

2801 AAATACGATT TATCTAATTT ACACGAAATT GCTTCTGGGG GCGCACCTCT TTCGAAAGAA GTCGGGGAAG

2871 CGGTTGCAAA ACGCTTCCAT CTTCCAGGGA TACGACAAGG ATATGGGCTC ACTGAGACTA CATCAGCTAT

2941 TCTGATTACA CCCGAGGGGG ATGATAAACC GGGCGCGGTC GGTAAAGTTG TTCCATTTTT TGAAGCGAAG

3011 GTTGTGGATC TGGATACCGG GAAAACGCTG GGCGTTAATC AGAGAGGCGA ATTATGTGTC AGAGGACCTA

3081 TGATTATGTC CGGTTATGTA AACAATCCGG AAGCGACCAA CGCCTTGATT GACAAGGATG GATGGCTACA

3151 TTCTGGAGAC ATAGCTTACT GGGACGAAGA CGAACACTTC TTCATAGTTG ACCGCTTGAA GTCTTTAATT

ClaI (3259)

3221 AAATACAAAG GATATCAGGT GGCCCCCGCT GAATTGGAAT CGATATTGTT ACAACACCCC AACATCTTCG

3291 ACGCGGGCGT GGCAGGTCTT CCCGACGATG ACGCCGGTGA ACTTCCCGCC GCCGTTGTTG TTTTGGAGCA

3361 CGGAAAGACG ATGACGGAAA AAGAGATCGT GGATTACGTC GCCAGTCAAG TAACAACCGC GAAAAAGTTG

3431 CGCGGAGGAG TTGTGTTTGT GGACGAAGTA CCGAAAGGTC TTACCGGAAA ACTCGACGCA AGAAAAATCA

ApaI (3557)
XhoI (3548) KpnI(3563)

3501 GAGAGATCCT CATAAAGGCC AAGAAGGGCG GAAAGTCCAA ATTGTAAcTC GAGGGGGGGC CCGGTACCTT

3571 TAAGACCAAT GACTTACAAG GCAGCTGTAG ATCTTAGCCA CTTTTTAAAA GAAAAGGGGG GACTGGAAGG

3641 GCTAATTCAC TCCCAAAGAA GACAAGATAT CCTTGATCTG TGGATCTACC ACACACAAGG CTACTTCCCT

3711 GATTGGCAGA ACTACACACC AGGGCCAGGG GTCAGATATC CACTGACCTT TGGATGGTGC TACAAGCTAG

3781 TACCAGTTGA GCCAGATAAG GTAGAAGAGG CCAATAAAGG AGAGAACACC AGCTTGTTAC ACCCTGTGAG

3851 CCTGCATGGA ATGGATGACC CTGAGAGAGA AGTGTTAGAG TGGAGGTTTG ACAGCCGCCT AGCATTTCAT

3921 CACGTGGCCC GAGAGCTGCA TCCGGAGTAC TTCAAGAACT GCTGACATCG AGCTTGCTAC AAGGGACTTT

3991 CCGCTGGGGA CTTTCCAGGG AGGCGTGGCC TGGGCGGGAC TGGGGAGTGG CGAGCCCTCA GATGCTGCAT

4061 ATAAGCAGCT GCTTTTTGCC TGTACTGGGT CTCTCTGGTT AGACCAGATC TGAGCCTGGG AGCTCTCTGG

4131 CTAACTAGGG AACCCACTGC TTAAGCCTCA ATAAAGCTTG CCTTGAGTGC TTCAAGTAGT GTGTGCCCGT

4201 CTGTTGTGTG ACTCTGGTAA CTAGAGATCC CTCAGACCCT TTTAGTCAGT GTGGAAAATC TCTAGCACCC

4271 CCCAGGAGGT AGAGGTTGCA GTGAGCCAAG ATCGCGCCAC TGCATTCCAG CCTGGGCAAG AAAACAAGAC
4341 TGTCTAAAAT AATAATAATA AGTTAAGGGT ATTAAATATA TTTATACATG GAGGTCATAA AAATATATAT
4411 ATTTGGGCTG GGCGCAGTGG CTCACACCTG CGCCCGGCCC TTTGGGAGGC CGAGGCAGGT GGATCACCTG
4481 AGTTTGGGAG TTCCAGACCA GCCTGACCAA CATGGAGAAA CCCCTTCTCT GTGTATTTTT AGTAGATTTT
4551 ATTTTATGTG TATTTTATTC ACAGGTATTT CTGGAAAACT GAAACTGTTT TTCCTCTACT CTGATACCAC
4621 AAGAATCATC AGCACAGAGG AAGACTTCTG TGATCAAATG TGGTGGGAGA GGGAGGTTTT CACCAGCACA
4691 TGAGCAGTCA GTTCTGCCGC AGACTCGGCG GGTGTCCTTC GGTTCAGTTC CAACACCGCC TGCCTGGAGA
4761 GAGGTCAGAC CACAGGGTGA GGGCTCAGTC CCCAAGACAT AAACACCCAA GACATAAACA CCCAACAGGT
4831 CCACCCCGCC TGCTGCCCAG GCAGAGCCGA TTCACCAAGA CGGGAATTAG GATAGAGAAA GAGTAAGTCA
4901 CACAGAGCCG GCTGTGCGGG AGAACGGAGT TCTATTATGA CTCAAATCAG TCTCCCCAAG CATTCGGGGA
4971 TCAGAGTTTT TAAGGATAAC TTAGTGTGTA GGGGGCCAGT GAGTTGGAGA TGAAAGCGTA GGGAGTCGAA
5041 GCTGTCCTTT TGCGCCGAGT CAGTTCCTGG GTGGGGGCCA CAAGATCGGA TGAGCCAGTT TATCAATCCG
5111 GGGGTGCCAG CTGATCCATG GAGTGCAGGG TCTGCAAAAT ATCTCAAGCA CTGATTGATC TTAGGTTTTA
5181 CAATAGTGAT GTTACCCCAG GAACAATTTG GGGAAGGTCA GAATCTTGTA GCCTGTAGCT GCATGACTCC
5251 TAAACCATAA TTTCTTTTTT GTTTTTTTTT TTTTATTTTT GAGACAGGGT CTCACTCTGT CACCTAGGCT
5321 GGAGTGCAGT GGTGCAATCA CAGCTCACTG CAGCCCCTAG AGCGGCCGCC ACCGCGGTGG AGCTCCAATT
5391 CGCCCTATAG TGAGTCGTAT TACAATTCAC TGGCCGTCGT TTTACAACGT CGTGACTGGG AAAACCCTGG
5461 CGTTACCCAA CTTAATCGCC TTGCAGCACA TCCCCCTTTC GCCAGCTGGC GTAATAGCGA AGAGGCCCGC
5531 ACCGATCGCC CTTCCCAACA GTTGCGCAGC CTGAATGGCG AATGGCGCGA AATTGTAAAC GTTAATATTT
5601 TGTTAAAATT CGCGTTAAAT TTTTGTTAAA TCAGCTCATT TTTTAACCAA TAGGCCGAAA TCGGCAAAAT
5671 CCCTTATAAA TCAAAAGAAT AGACCGAGAT AGGGTTGAGT GTTGTTCCAG TTTGGAACAA GAGTCCACTA
5741 TTAAAGAACG TGGACTCCAA CGTCAAAGGG CGAAAAACCG TCTATCAGGG CGATGGCCCA CTACGTGAAC
5811 CATCACCCTA ATCAAGTTTT TTGGGGTCGA GGTGCCGTAA AGCACTAAAT CGGAACCCTA AAGGGAGCCC
5881 CCGATTTAGA GCTTGACGGG GAAAGCCGGC GAACGTGGCG AGAAAGGAAG GGAAGAAAGC GAAAGGAGCG
5951 GGCGCTAGGG CGCTGGCAAG TGTAGCGGTC ACGCTGCGCG TAACCACCAC ACCCGCCGCG CTTAATGCGC
6021 CGCTACAGGG CGCGTCCCAG GTGGCACTTT TCGGGGAAAT GTGCGCGGAA CCCCTATTTG TTTATTTTTC
6091 TAAATACATT CAAATATGTA TCCGCTCATG AGACAATAAC CCTGATAAAT GCTTCAATAA TATTGAAAAA

```
6161  GGAAGAGTAT GAGTATTCAA CATTTCCGTG TCGCCCTTAT TCCCTTTTTT GCGGCATTTT GCCTTCCTGT
6231  TTTTGCTCAC CCAGAAACGC TGGTGAAAGT AAAAGATGCT GAAGATCAGT TGGGTGCACG AGTGGGTTAC
6301  ATCGAACTGG ATCTCAACAG CGGTAAGATC CTTGAGAGTT TTCGCCCCGA AGAACGTTTT CCAATGATGA
6371  GCACTTTTAA AGTTCTGCTA TGTGGCGCGG TATTATCCCG TATTGACGCC GGGCAAGAGC AACTCGGTCG
6441  CCGCATACAC TATTCTCAGA ATGACTTGGT TGAGTACTCA CCAGTCACAG AAAAGCATCT TACGGATGGC
6511  ATGACAGTAA GAGAATTATG CAGTGCTGCC ATAACCATGA GTGATAACAC TGCGGCCAAC TTACTTCTGA
6581  CAACGATCGG AGGACCGAAG GAGCTAACCG CTTTTTTGCA CAACATGGGG GATCATGTAA CTCGCCTTGA
6651  TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT ACCAAACGAC GAGCGTGACA CCACGATGCC TGTAGCAATG
6721  GCAACAACGT TGCGCAAACT ATTAACTGGC GAACTACTTA CTCTAGCTTC CCGGCAACAA TTAATAGACT
6791  GGATGGAGGC GGATAAAGTT GCAGGACCAC TTCTGCGCTC GGCCCTTCCG GCTGGCTGGT TTATTGCTGA
6861  TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CGGTATCATT GCAGCACTGG GGCCAGATGG TAAGCCCTCC
6931  CGTATCGTAG TTATCTACAC GACGGGGAGT CAGGCAACTA TGGATGAACG AAATAGACAG ATCGCTGAGA
7001  TAGGTGCCTC ACTGATTAAG CATTGGTAAC TGTCAGACCA AGTTTACTCA TATATACTTT AGATTGATTT
7071  AAAACTTCAT TTTTAATTTA AAAGGATCTA GGTGAAGATC CTTTTTGATA ATCTCATGAC CAAAATCCCT
7141  TAACGTGAGT TTTCGTTCCA CTGAGCGTCA GACCCCGTAG AAAAGATCAA AGGATCTTCT TGAGATCCTT
7211  TTTTTCTGCG CGTAATCTGC TGCTTGCAAA CAAAAAAACC ACCGCTACCA GCGGTGGTTT GTTTGCCGGA
7281  TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC AGCAGAGCGC AGATACCAAA TACTGTCCTT
7351  CTAGTGTAGC CGTAGTTAGG CCACCACTTC AAGAACTCTG TAGCACCGCC TACATACCTC GCTCTGCTAA
7421  TCCTGTTACC AGTGGCTGCT GCCAGTGGCG ATAAGTCGTG TCTTACCGGG TTGGACTCAA GACGATAGTT
7491  ACCGGATAAG GCGCAGCGGT CGGGCTGAAC GGGGGGTTCG TGCACACAGC CCAGCTTGGA GCGAACGACC
7561  TACACCGAAC TGAGATACCT ACAGCGTGAG CTATGAGAAA GCGCCACGCT TCCCGAAGGG AGAAAGGCGG
7631  ACAGGTATCC GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG CACGAGGGAG CTTCCAGGGG GAAACGCCTG
7701  GTATCTTTAT AGTCCTGTCG GGTTTCGCCA CCTCTGACTT GAGCGTCGAT TTTTGTGATG CTCGTCAGGG
7771  GGGCGGAGCC TATGGAAAAA CGCCAGCAAC GCGGCCTTTT TACGGTTCCT GGCCTTTTGC TGGCCTTTTG
7841  CTCACATGTT CTTTCCTGCG TTATCCCCTG ATTCTGTGGA TAACCGTATT ACCGCCTTTG AGTGAGCTGA
7911  TACCGCTCGC CGCAGCCGAA CGACCGAGCG CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA GCGCCCAATA
7981  CGCAAACCGC CTCTCCCCGC GCGTTGGCCG ATTCATTAAT GCAGCTGGCA CGACAGGTTT CCCGACTGGA
8051  AAGCGGGCAG TGAGCGCAAC GCAATTAATG TGAGTTAGCT CACTCATTAG GCACCCCAGG CTTTACACTT
8121  TATGCTTCCG GCTCGTATGT TGTGTGGAAT TGTGAGCGGA TAACAATTTC ACACAGGAAA CAGCTATGAC
8191  CATGATTACG CCAAGCTCGG AATTAACCCT CACTAAAGGG AACAAAAGCT GCTGCAGGGT CCCTAACTGC
8261  CAAGCCCCAC AGTGTGCCCT GAGGCTGCCC CTTCCTTCTA GCGGCTGCCC CCACTCGGCT TTGCTTTCCC
8331  TAGTTTCAGT TACTTGCGTT CAGCCAAGGT CTGAAACTAG GTGCGCACAG AGCGGTAAGA CTGCGAGAGA
8401  AAGAGACCAG CTTTACAGGG GGTTTATCAC AGTGCACCCT GACAGTCGTC AGCCTCACAG GGGGTTTATC
8471  ACATTGCACC CTGACAGTCG TCAGCCTCAC AGGGGGTTTA TCACAGTGCA CCCTTACAAT CATTCCATTT
8541  GATTCACAAT TTTTTTAGTC TCTACTGTGC CTAACTTGTA AGTTAAATTT GATCAGAGGT GTGTTCCCAG
8611  AGGGGAAAAC AGTATATACA GGGTTCAGTA CTATCGCATT TCAGGCCTCC ACCTGGGTCT TGGAATGTGT
8681  CCCCCGAGGG GTGATGACTA CCTCAGTTGG ATCTCCACAG GTCACAGTGA CACAAGATAA CCAAGACACC
8751  TCCCAAGGCT ACCACAATGG GCCGCCCTCC ACGTGCACAT GGCCGGAGGA ACTGCCATGT CGGAGGTGCA
8821  AGCACACCTG CGCATCAGAG TCCTTGGTGT GGAGGGAGGG ACCAGCGCAG CTTCCAGCCA TCCACCTGAT
8891  GAACAGAACC TAGGGAAAGC CCCAGTTCTA CTTACACCAG GAAAGGC
```

```
mBCwCN frag   ---------- ---------- --------C- --AC---G-- ----------
m2BCwCN frag  ---------- ---------- --------C- ---G---G-- ----------
BC/HXB2       ---------- ---------- ---------- ---------- ----------
BC/NL43       ---------- ---------- ---------- ---------- ----------
#1            ..........................................................
              CGCGCACGGC AAGAGGCGAG GGGCGGCGAC TGGTGAGTAC GCCAAAAATT

mBCwCN frag   ---------- ---------- --------C- C--------- ----------
m2BCwCN frag  ---------- ---------- ---------- ---------- ----------
BC/HXB2       -------T-- ---------- ---------- ---------- ----------
BC/NL43       ---------- ---------- ---------- ---------- -----G----
#51           ..........................................................
              TTGACTAGCG GAGGCTAGAA GGAGAGAGAT GGGTGCGAGA GCGTCAGTAT

mBCwCN frag   ---------- ---------- ---------- ---------- ----------
m2BCwCN frag  ---------- ---------- ---------- ---------- ----------
BC/HXB2       ---------- ---------- ---------- ---------- ----------
BC/NL43       ---------- ---------- AA-------- ---------- ----------
#101          ..........................................................
              TAAGCGGGGG AGAATTAGAT CG
```

FIG. 12

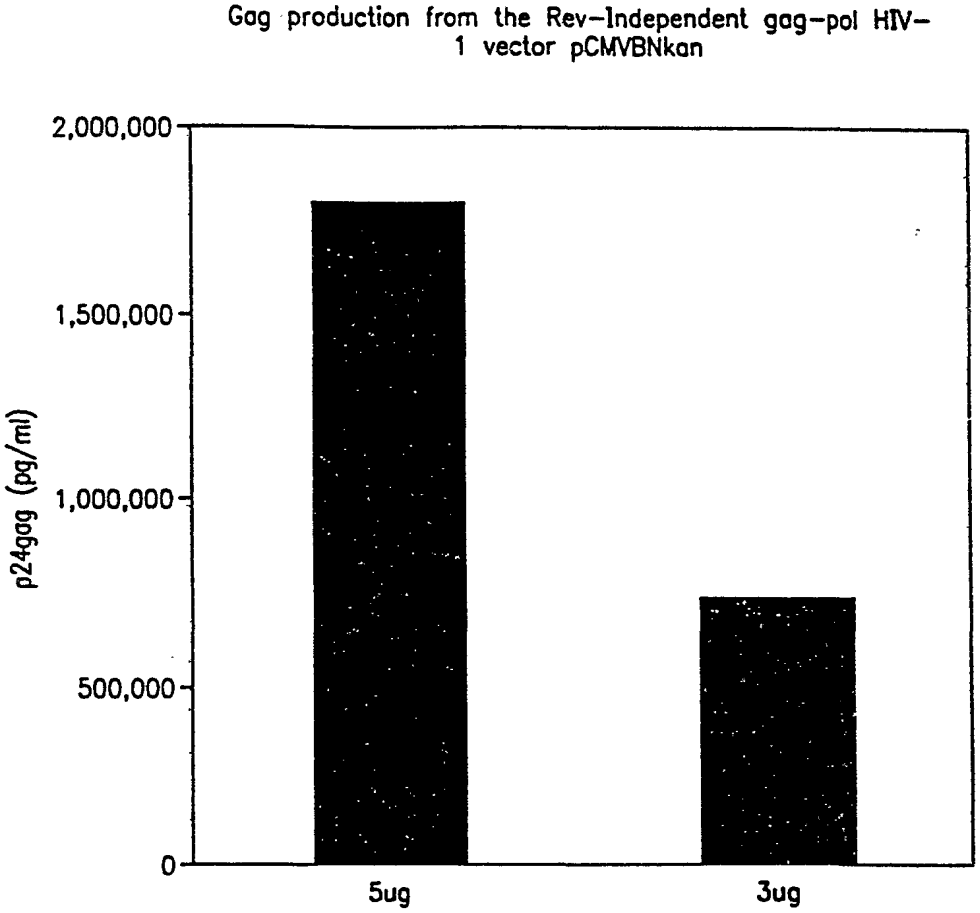

Gag production from the Rev-Independent gag-pol HIV-1 vector pCMVBNkan
2,000,000
1,500,000
1,000,000
500,000
0
p24gag (pg/ml)
5ug
3ug

FIG. 13

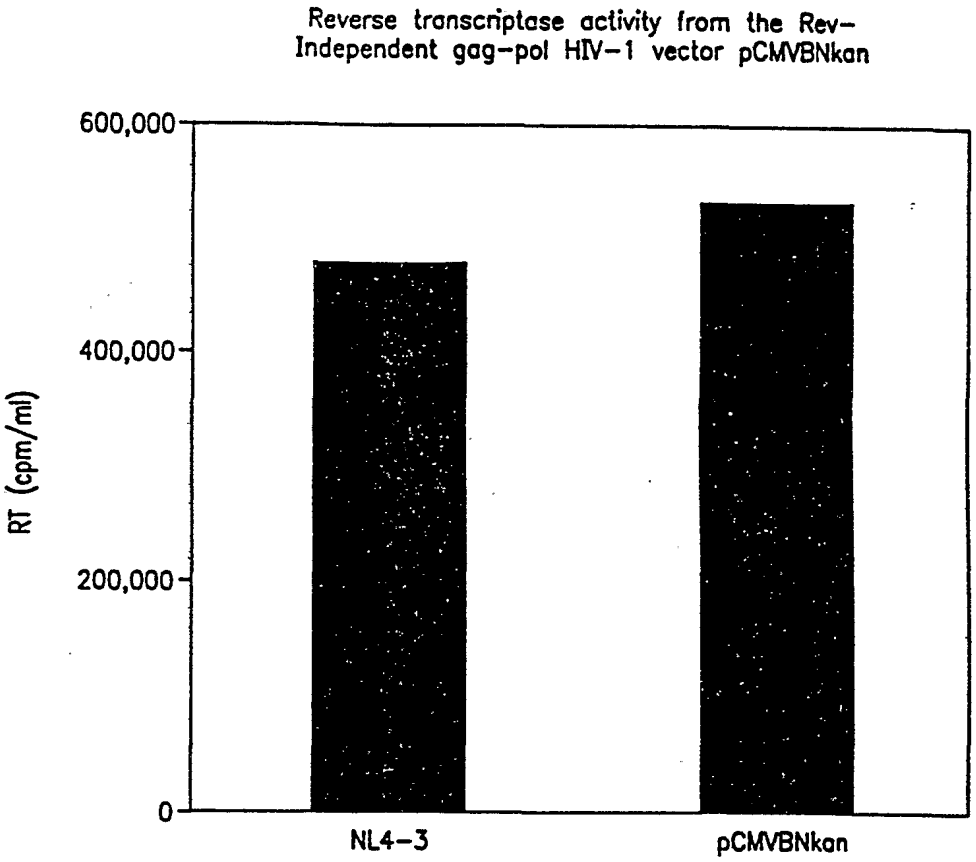

Reverse transcriptase activity from the Rev-Independent gag-pol HIV-1 vector pCMVBNkan
600,000
400,000
200,000
0
RT (cpm/ml)
NL4-3
pCMVBNkan

FIG. 14

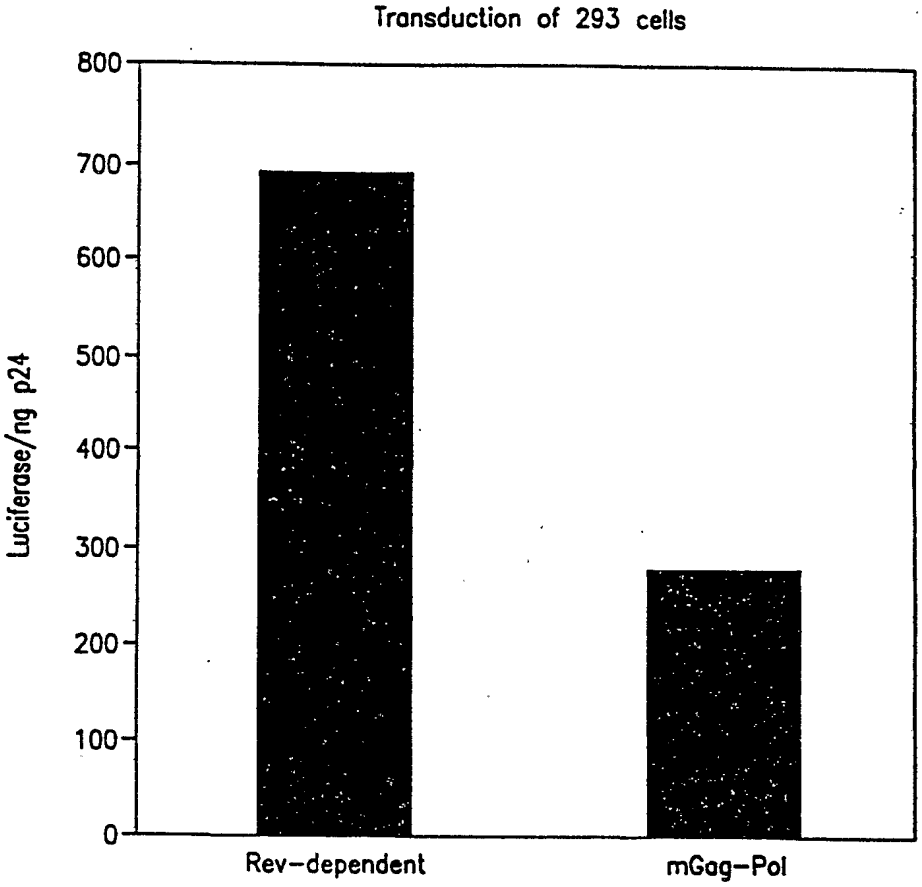

Transduction of 293 cells
Luciferase/ng p24
800
700
600
500
400
300
200
100
0
Rev-dependent
mGag-Pol

FIG. 15A

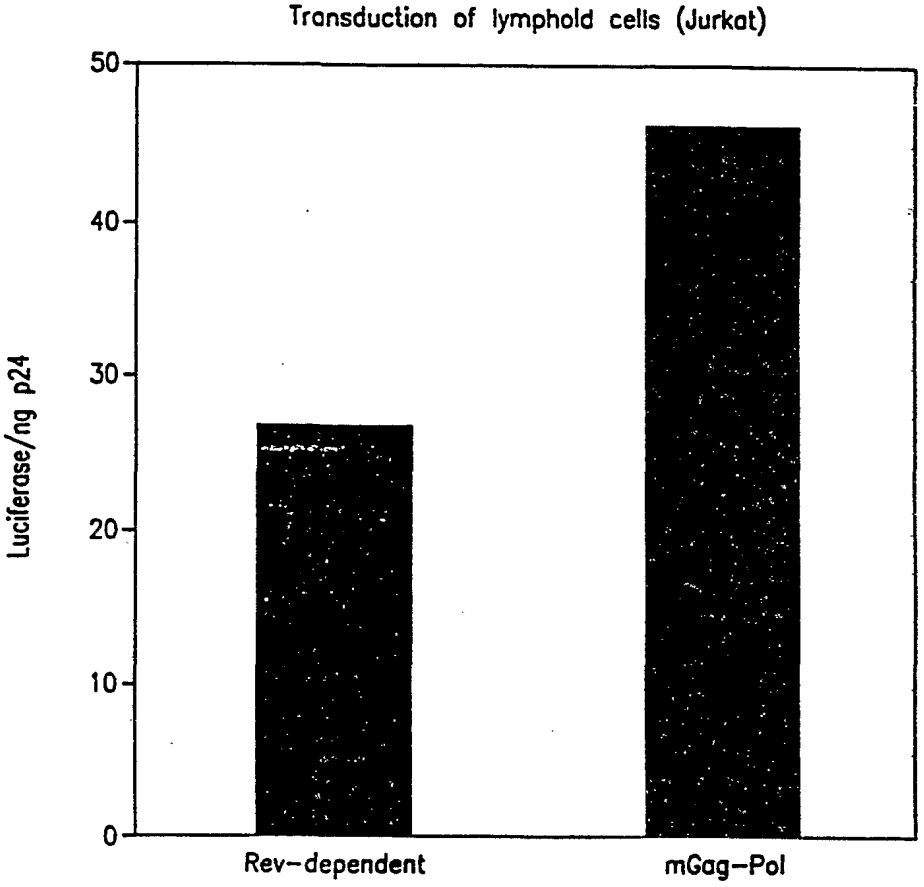

Transduction of lymphoid cells (Jurkat)
Luciferase/ng p24
50
40
30
20
10
0
Rev-dependent
mGag-Pol

FIG. 15B

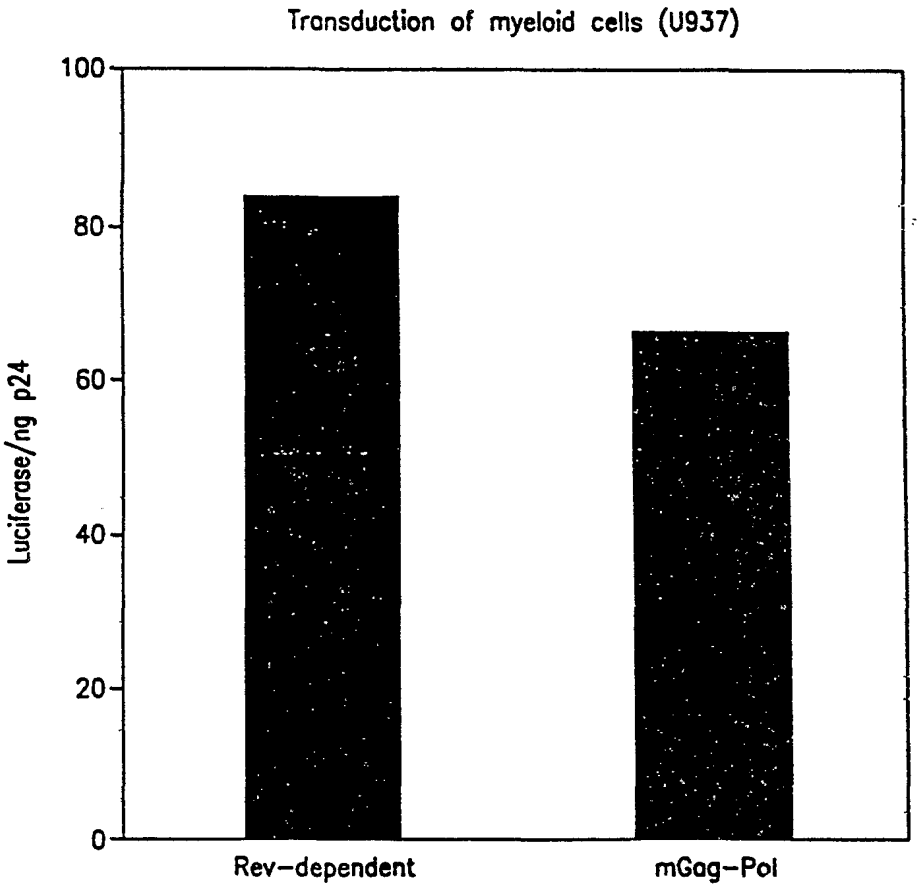

Transduction of myeloid cells (U937)
Luciferase/ng p24
100
80
60
40
20
0
Rev-dependent
mGag-Pol

FIG. 15C

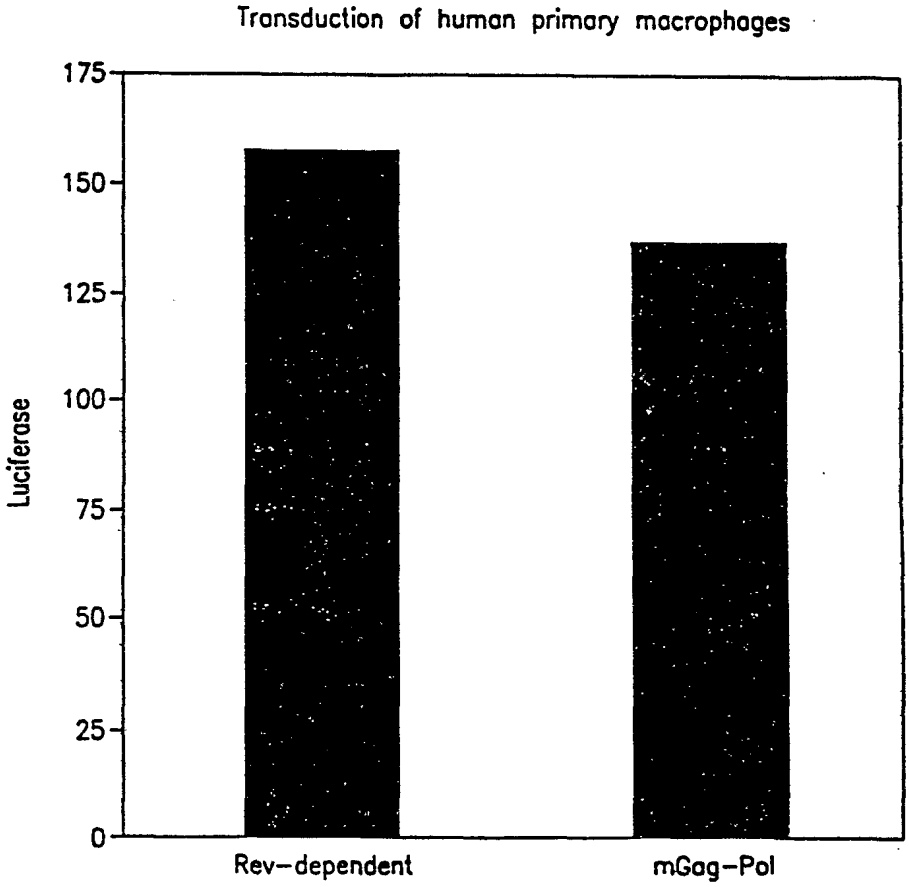

Transduction of human primary macrophages
Luciferase
175
150
125
100
75
50
25
0
Rev-dependent
mGag-Pol

FIG. 15D

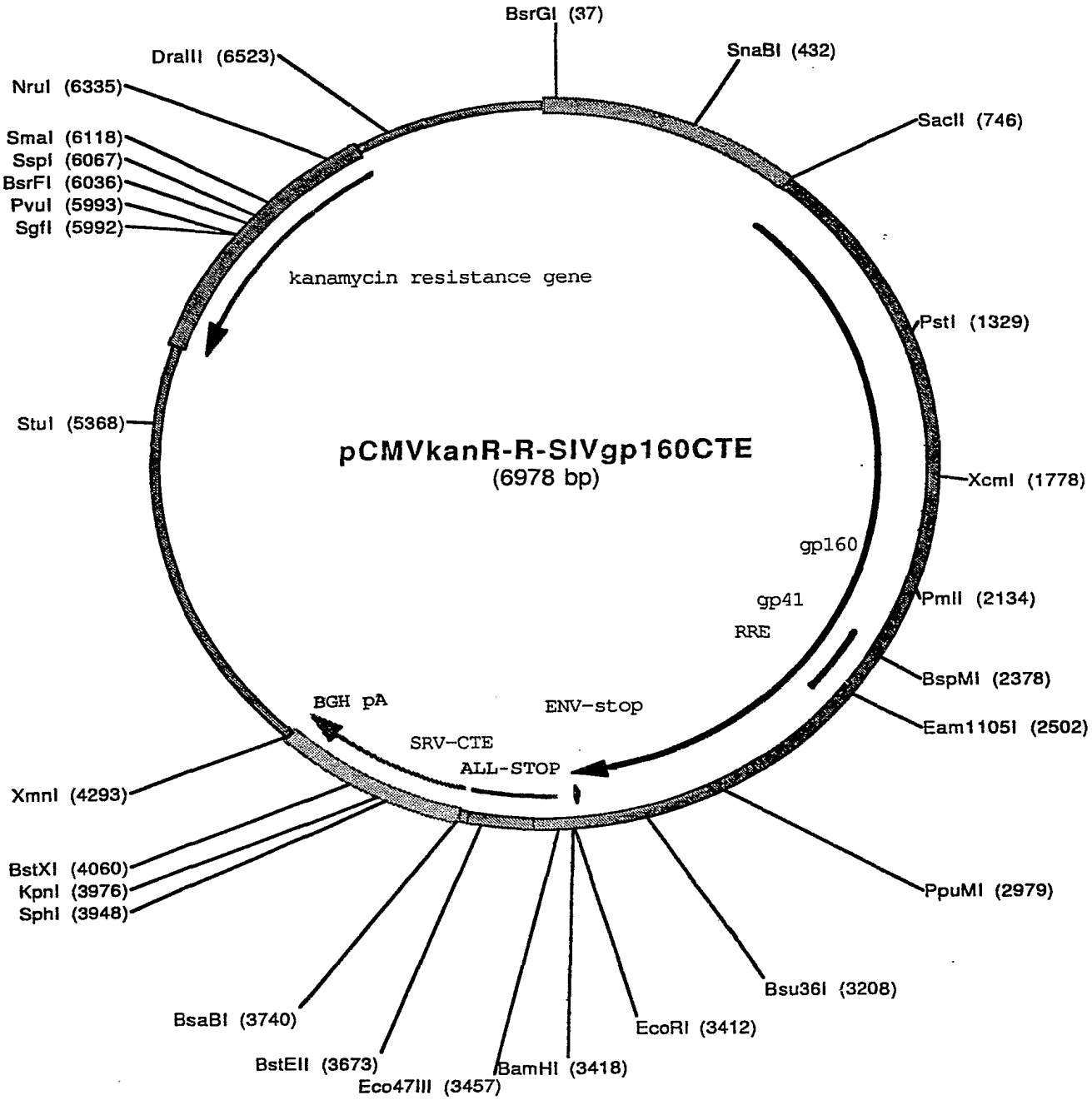

BsrGI (37)
SnaBI (432)
SacII (746)
PstI (1329)
XcmI (1778)
PmlI (2134)
BspMI (2378)
Eam1105I (2502)
PpuMI (2979)
Bsu36I (3208)
EcoRI (3412)
BamHI (3418)
Eco47III (3457)
BstEII (3673)
BsaBI (3740)
BstXI (4060)
KpnI (3976)
SphI (3948)
XmnI (4293)
StuI (5368)
NruI (6335)
DraIII (6523)
SmaI (6118)
SspI (6067)
BsrFI (6036)
PvuI (5993)
SgfI (5992)
kanamycin resistance gene
pCMVkanR-R-SIVgp160CTE
(6978 bp)
gp160
gp41
RRE
ENV-stop
ALL-STOP
SRV-CTE
BGH pA

FIG. 16

BsrGI (37)

1 CCTGGCCATTGCATACGTTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCCATGTTGA
81 CATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTAC
161 ATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCA
241 TAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAA
321 GTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGAC

SnaBI (432)

401 CTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACA
481 TCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGG
561 CACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTG
641 GGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATA

SacII (746)

721 GAAGACACCGGGACCGATCCAGCCTCCGCGGCCGCGCTAAGTATGGGATGTCTTGGGAATCAGCTGCTTATCGCCATCT

1▶MetGlyCysLeuGlyAsnGlnLeuLeuIleAlaIleL
801 TGCTTTTAAGTGTCTATGGGATCTATTGTACTCTATATGTCACAGTCTTTTATGGTGTACCAGCTTGGAGGAATGCGACA

13▶euLeuLeuSerValTyrGlyIleTyrCysThrLeuTyrValThrValPheTyrGlyValProAlaTrpArgAsnAlaThr
881 ATTCCCCTCTTTTGTGCAACCAAGAATAGGGATACTTGGGGAACAACTCAGTGCCTACCAGATAATGGTGATTATTCAGA

40▶IleProLeuPheCysAlaThrLysAsnArgAspThrTrpGlyThrThrGlnCysLeuProAspAsnGlyAspTyrSerGl
961 AGTGGCCCTTAATGTTACAGAAAGCTTTGATGCCTGGAATAATACAGTCACAGAACAGGCAATAGAGGATGTATGGCAAC

66▶uValAlaLeuAsnValThrGluSerPheAspAlaTrpAsnAsnThrValThrGluGlnAlaIleGluAspValTrpGlnL
1041 TCTTTGAGACCTCAATAAAGCCTTGTGTAAAATTATCCCCATTATGCATTACTATGAGATGCAATAAAAGTGAGACAGAT

93▶euPheGluThrSerIleLysProCysValLysLeuSerProLeuCysIleThrMetArgCysAsnLysSerGluThrAsp
1121 AGATGGGGATTGACAAAATCAATAACAACAACAGCATCAACAACATCAACGACAGCATCAGCAAAAGTAGACATGGTCAA

120▶ArgTrpGlyLeuThrLysSerIleThrThrThrAlaSerThrThrSerThrThrAlaSerAlaLysValAspMetValAs
1201 TGAGACTAGTTCTTGTATAGCCCAGGATAATTGCACAGGCTTGGAACAAGAGCAAATGATAAGCTGTAAATTCAACATGA

146▶nGluThrSerSerCysIleAlaGlnAspAsnCysThrGlyLeuGluGlnGluGlnMetIleSerCysLysPheAsnMetT

PstI (1329)

1281 CAGGGTTAAAAAGAGACAAGAAAAAAGAGTACAATGAAACTTGGTACTCTGCAGATTTGGTATGTGAACAAGGGAATAAC

173▶hrGlyLeuLysArgAspLysLysLysGluTyrAsnGluThrTrpTyrSerAlaAspLeuValCysGluGlnGlyAsnAsn
1361 ACTGGTAATGAAAGTAGATGTTACATGAACCACTGTAACACTTCTGTTATCCAAGAGTCTTGTGACAAACATTATTGGGA

200▶ThrGlyAsnGluSerArgCysTyrMetAsnHisCysAsnThrSerValIleGlnGluSerCysAspLysHisTyrTrpAs
1441 TGCTATTAGATTTAGGTATTGTGCACCTCCAGGTTATGCTTTGCTTAGATGTAATGACACAAATTATTCAGGCTTTATGC

226▶pAlaIleArgPheArgTyrCysAlaProProGlyTyrAlaLeuLeuArgCysAsnAspThrAsnTyrSerGlyPheMetP
1521 CTAAATGTTCTAAGGTGGTGGTCTCTTCATGCACAAGGATGATGGAGACACAGACTTCTACTTGGTTTGGCTTTAATGGA

253▶roLysCysSerLysValValValSerSerCysThrArgMetMetGluThrGlnThrSerThrTrpPheGlyPheAsnGly
1601 ACTAGAGCAGAAAATAGAACTTATATTTACTGGCATGGTAGGGATAATAGGACTATAATTAGTTTAAATAAGTATTATAA

280▶ThrArgAlaGluAsnArgThrTyrIleTyrTrpHisGlyArgAspAsnArgThrIleIleSerLeuAsnLysTyrTyrAs
1681 TCTAACAATGAAATGTAGAAGACCAGGAAATAAGACAGTTTTACCAGTCACCATTATGTCTGGATTGGTTTTCCACTCAC

306▶nLeuThrMetLysCysArgArgProGlyAsnLysThrValLeuProValThrIleMetSerGlyLeuValPheHisSerG

XcmI (1778)

1761 AACCAATCAATGATAGGCCAAAGCAGGCATGGTGTTGGTTTGGAGGAAAATGGAAGGATGCAATAAAAGAGGTGAAGCAG

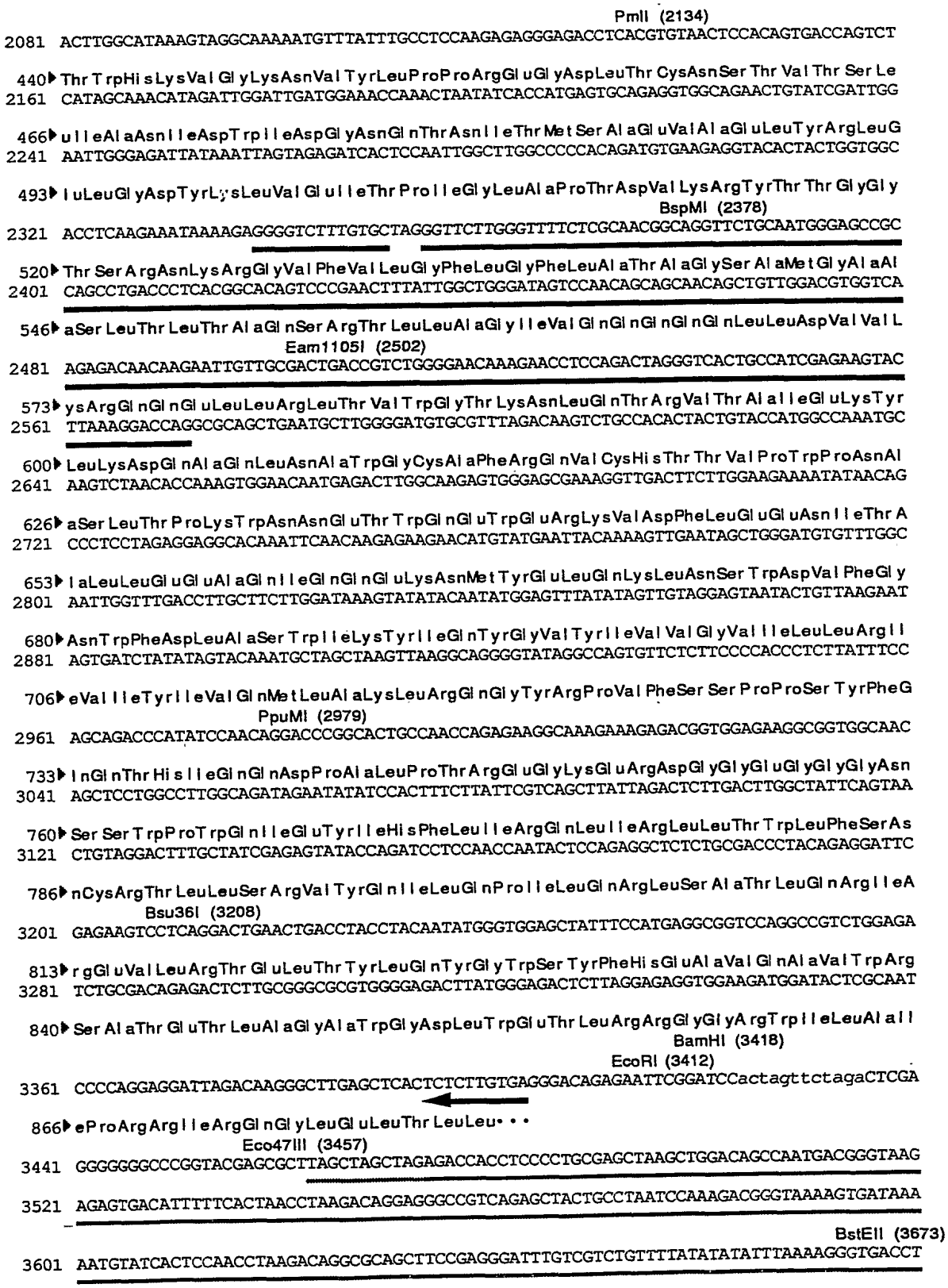
333▶InProIleAsnAspArgProLysGlnAlaTrpCysTrpPheGlyGlyLysTrpLysAspAlaIleLysGluValLysGln
1841 ACCATTGTCAAACATCCCAGGTATACTGGAACTAACAATACTGATAAAATCAATTTGACGGCTCCTGGAGGAGGAGATCC

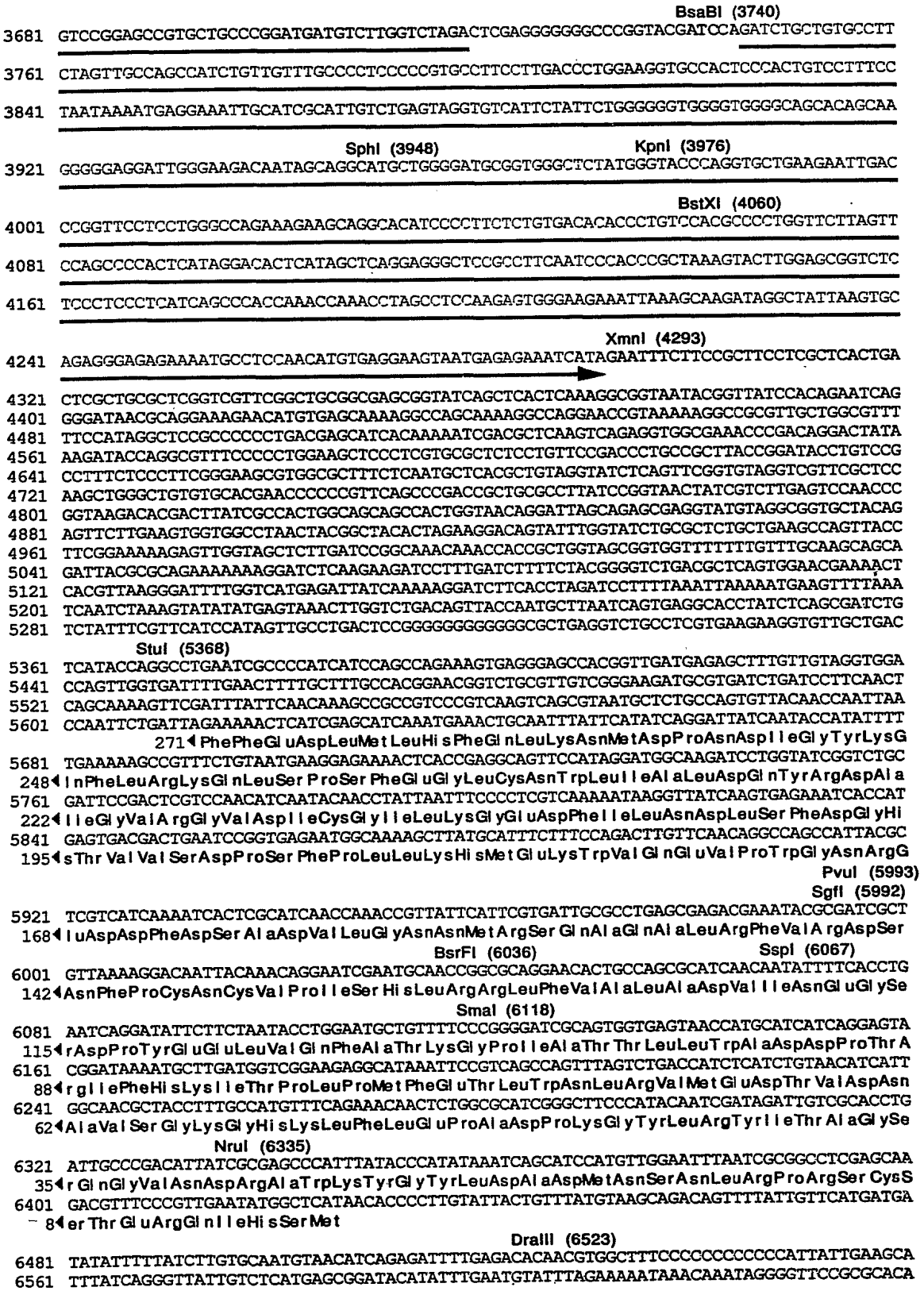
360▶ThrIleValLysHisProArgTyrThrGlyThrAsnAsnThrAspLysIleAsnLeuThrAlaProGlyGlyGlyAspPr
1921 GGAAGTTACCTTCATGTGGACAAATTGCAGAGGAGAGTTCCTCTACTGTAAAATGAATTGGTTTCTAAATTGGGTAGAAG

386▶oGluValThrPheMetTrpThrAsnCysArgGlyGluPheLeuTyrCysLysMetAsnTrpPheLeuAsnTrpValGluA
2001 ATAGGAATACAGCTAACCAGAAGCCAAAGGAACAGCATAAAAGGAATTACGTGCCATGTCATATTAGACAAATAATCAAC

413▶spArgAsnThrAlaAsnGlnLysProLysGluGlnHisLysArgAsnTyrValProCysHisIleArgGlnIleIleAsn

FIG. 17


PmlI (2134)
2081 ACTTGGCATAAAGTAGGCAAAAATGTTTATTTGCCTCCAAGAGAGGGAGACCTCACGTGTAACTCCACAGTGACCAGTCT
440 Thr TrpHisLysValGlyLysAsnValTyrLeuProProArgGluGlyAspLeuThr CysAsnSer Thr Val Thr Ser Le
2161 CATAGCAAACATAGATTGGATTGATGGAAACCAAACTAATATCACCATGAGTGCAGAGGTGGCAGAACTGTATCGATTGG
466 uIleAlaAsnIleAspTrpIleAspGlyAsnGlnThrAsnIleThrMetSer AlaGluValAlaGluLeuTyrArgLeuG
2241 AATTGGGAGATTATAAATTAGTAGAGATCACTCCAATTGGCTTGGCCCCCACAGATGTGAAGAGGTACACTACTGGTGGC
493 luLeuGlyAspTyrLysLeuValGluIleThr ProIleGlyLeuAlaProThrAspVal LysArgTyrThr Thr GlyGly
BspMI (2378)
2321 ACCTCAAGAAATAAAAGAGGGGTCTTTGTGCTAGGGTTCTTGGGTTTTCTCGCAACGGCAGGTTCTGCAATGGGAGCCGC
520 Thr Ser ArgAsnLysArgGlyVal PheVal LeuGlyPheLeuGlyPheLeuAlaThr AlaGlySer AlaMetGlyAlaAl
2401 CAGCCTGACCCTCACGGCACAGTCCCGAACTTTATTGGCTGGGATAGTCCAACAGCAGCAACAGCTGTTGGACGTGGTCA
546 aSer LeuThr LeuThr AlaGlnSer ArgThr LeuLeuAlaGlyIleVal GlnGlnGlnGlnLeuLeuAspVal Val L
Eam1105I (2502)
2481 AGAGACAACAAGAATTGTTGCGACTGACCGTCTGGGGAACAAAGAACCTCCAGACTAGGGTCACTGCCATCGAGAAGTAC
573 ysArgGlnGlnGluLeuLeuArgLeuThr Val TrpGlyThr LysAsnLeuGlnThr ArgVal Thr AlaIleGluLysTyr
2561 TTAAAGGACCAGGCGCAGCTGAATGCTTGGGGATGTGCGTTTAGACAAGTCTGCCACACTACTGTACCATGGCCAAATGC
600 LeuLysAspGlnAlaGlnLeuAsnAlaTrpGlyCysAlaPheArgGlnVal CysHisThr Thr Val ProTrpProAsnAl
2641 AAGTCTAACACCAAAGTGGAACAATGAGACTTGGCAAGAGTGGGAGCGAAAGGTTGACTTCTTGGAAGAAAATATAACAG
626 aSer LeuThr ProLysTrpAsnAsnGluThr TrpGlnGluTrpGluArgLysVal AspPheLeuGluGluAsnIleThr A
2721 CCCTCCTAGAGGAGGCACAAATTCAACAAGAGAAGAACATGTATGAATTACAAAAGTTGAATAGCTGGGATGTGTTTGGC
653 laLeuLeuGluGluAlaGlnIleGlnGlnGluLysAsnMetTyrGluLeuGlnLysLeuAsnSer TrpAspVal PheGly
2801 AATTGGTTTGACCTTGCTTCTTGGATAAAGTATATACAATATGGAGTTTATATAGTTGTAGGAGTAATACTGTTAAGAAT
680 AsnTrpPheAspLeuAlaSer TrpIleLysTyrIleGlnTyrGlyValTyrIleVal ValGlyValIleLeuLeuArgIl
2881 AGTGATCTATATAGTACAAATGCTAGCTAAGTTAAGGCAGGGGTATAGGCCAGTGTTCTCTTCCCCACCCTCTTATTTCC
706 eValIleTyrIleValGlnMetLeuAlaLysLeuArgGlnGlyTyrArgProVal PheSer Ser ProProSer TyrPheG
PpuMI (2979)
2961 AGCAGACCCATATCCAACAGGACCCGGCACTGCCAACCAGAGAAGGCAAAGAAAGAGACGGTGGAGAAGGCGGTGGCAAC
733 InGlnThrHisIleGlnGlnAspProAlaLeuProThrArgGluGlyLysGluArgAspGlyGlyGluGlyGlyAsn
3041 AGCTCCTGGCCTTGGCAGATAGAATATATCCACTTTCTTATTCGTCAGCTTATTAGACTCTTGACTTGGCTATTCAGTAA
760 Ser Ser TrpProTrpGlnIleGluTyrIleHisPheLeuIleArgGlnLeuIleArgLeuLeuThr TrpLeuPheSerAs
3121 CTGTAGGACTTTGCTATCGAGAGTATACCAGATCCTCCAACCAATACTCCAGAGGCTCTCTGCGACCCTACAGAGGATTC
786 nCysArgThr LeuLeuSer ArgVal TyrGlnIleLeuGlnProIleLeuGlnArgLeuSer AlaThr LeuGlnArgIleA
Bsu36I (3208)
3201 GAGAAGTCCTCAGGACTGAACTGACCTACCTACAATATGGGTGGAGCTATTTCCATGAGGCGGTCCAGGCCGTCTGGAGA
813 rgGluVal LeuArgThr GluLeuThr TyrLeuGlnTyrGlyTrpSer TyrPheHisGluAlaVal GlnAlaVal TrpArg
3281 TCTGCGACAGAGACTCTTGCGGGCGCGTGGGGAGACTTATGGGAGACTCTTAGGAGAGGTGGAAGATGGATACTCGCAAT
840 Ser AlaThr GluThr LeuAlaGlyAlaTrpGlyAspLeuTrpGluThr LeuArgArgGlyGlyArgTrpIleLeuAlaIl
BamHI (3418)
EcoRI (3412)
3361 CCCCAGGAGGATTAGACAAGGGCTTGAGCTCACTCTCTTGTGAGGGACAGAGAATTCGGATCCactagttctagaCTCGA
866 eProArgArgIleArgGlnGlyLeuGluLeuThr LeuLeu• • •
Eco47III (3457)
3441 GGGGGGGCCCGGTACGAGCGCTTAGCTAGCTAGAGACCACCTCCCCTGCGAGCTAAGCTGGACAGCCAATGACGGGTAAG
3521 AGAGTGACATTTTTCACTAACCTAAGACAGGAGGGCCGTCAGAGCTACTGCCTAATCCAAAGACGGGTAAAAGTGATAAA
BstEII (3673)
3601 AATGTATCACTCCAACCTAAGACAGGCGCAGCTTCCGAGGGATTTGTCGTCTGTTTTATATATATTTAAAAGGGTGACCT


BsaBI (3740)
SphI (3948)
KpnI (3976)
BstXI (4060)
XmnI (4293)
StuI (5368)
BsrFI (6036)
SspI (6067)
SmaI (6118)
NruI (6335)
DraIII (6523)
PvuI (5993)
SgfI (5992)

6641 TTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAG
6721 GCCCTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTC
6801 TGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTAT
6881 GCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCG
6961 CATCAGATTGGCTATTGG

SEQUENCE LISTING

<110> The Government of the United States of America, as

<120> MOLECULAR CLONES WITH MUTATED HIV GAG/POL, SIV GAG AND SIV ENV GENES

<130> 2026-4287PC2 HIV GAG/POL,SIV GAG & ENV

<140> TO BE ASSIGNED
<141> 2002-05-31

<150> 09/872,733
<151> 2001-06-01

<160> 19

<170> PatentIn Ver. 2.1

<210> 1
<211> 4338
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Mutated Human Immunodeficiency Virus - 1 Gag/Pol gene

<400> 1
atgggtgcga gagcgtcagt attaagcggg ggagaattag atcgatggga aaaaattcgg 60
ttaaggccag ggggaaagaa gtacaagcta aagcacatcg tatgggcaag cagggagcta 120
gaacgattcg cagttaatcc tggcctgtta gaaacatcag aaggctgtag acaaatactg 180
ggacagctac aaccatccct tcagacagga tcagaggagc ttcgatcact atacaacaca 240
gtagcaaccc tctattgtgt gcaccagcgg atcgagatca aggacaccaa ggaagcttta 300
gacaagatag aggaagagca aaacaagtcc aagaagaagg cccagcaggc agcagctgac 360
acaggacaca gcaatcaggt cagccaaaat taccctatag tgcagaacat ccaggggcaa 420
atggtacatc aggccatatc acctagaact ttaaatgcat gggtaaaagt agtagaagag 480
aaggctttca gcccagaagt gatacccatg ttttcagcat tatcagaagg agccacccca 540
caggacctga acacgatgtt gaacaccgtg gggggacatc aagcagccat gcaaatgtta 600
aaagagacca tcaatgagga agctgcagaa tgggatagag tgcatccagt gcatgcaggg 660
cctattgcac caggccagat gagagaacca aggggaagtg acatagcagg aactactagt 720
acccttcagg aacaaatagg atggatgaca aataatccac ctatcccagt aggagagatc 780
tacaagaggt ggataatcct gggattgaac aagatcgtga ggatgtatag ccctaccagc 840
attctggaca taagacaagg accaaaggaa ccctttagag actatgtaga ccggttctat 900
aaaactctaa gagctgagca agcttcacag gaggtaaaaa attggatgac agaaaccttg 960
ttggtccaaa atgcgaaccc agattgtaag accatcctga aggctctcgg cccagcggct 1020
acactagaag aaatgatgac agcatgtcag ggagtaggag gacccggcca taaggcaaga 1080
gttttggccg aggcgatgag ccaggtgacg aactcggcga ccataatgat gcagagaggc 1140

aacttccgga accagcggaa gatcgtcaag tgcttcaatt gtggcaaaga agggcacacc 1200
gccaggaact gccgggcccc ccggaagaag ggctgttgga aatgtggaaa ggaaggacac 1260
caaatgaaag attgtactga gagacaggct aatttttag ggaagatctg gccttcctac 1320
aagggaaggc cagggaattt tcttcagagc agaccagagc caacagcccc accagaagag 1380
agcttcaggt ctggggtaga gacaacaact ccccctcaga agcaggagcc gatagacaag 1440
gaactgtatc ctttaacttc cctcagatca ctctttggca acgacccctc gtcacagtaa 1500
ggatcggggg gcaactcaag gaagcgctgc tcgatacagg agcagatgat acagtattag 1560
aagaaatgag tttgccagga agatggaaac caaaaatgat agggggggatc gggggcttca 1620
tcaaggtgag gcagtacgac cagatactca tagaaatctg tggacataaa gctataggta 1680
cagtattagt aggacctacc tacacctgtc aacataattg gaagaaatct gttgacccag 1740
atcggctgca ccttgaactt ccccatcagc cctattgaga cggtgcccgt gaagttgaag 1800
ccggggatgg acggccccaa ggtcaagcaa tggccattga cgaaagagaa gatcaaggcc 1860
ttagtcgaaa tctgtacaga gatggagaag gaagggaaga tcagcaagat cgggcctgag 1920
aacccctaca acactccagt cttcgcaatc aagaagaagg acagtaccaa gtggagaaag 1980
ctggtggact tcagagagct gaacaagaga actcaggact tctgggaagt tcagctgggc 2040
atcccacatc ccgctgggtt gaagaagaag aagtcagtga cagtgctgga tgtgggtgat 2100
gcctacttct ccgttccctt ggacgaggac ttcaggaagt acactgcctt cacgatacct 2160
agcatcaaca acgagacacc aggcatccgc taccagtaca acgtgctgcc acagggatgg 2220
aagggatcac cagccatctt tcaaagcagc atgaccaaga tcctggagcc cttccgcaag 2280
caaaacccag acatcgtgat ctatcagtac atggacgacc tctacgtagg aagtgacctg 2340
gagatcgggg cagcacagga ccaagatcga ggagctgaga cagcatctgt tgaggtgggg 2400
actgaccaca ccagacaaga agcaccagaa ggaacctccc ttcctgtgga tgggctacga 2460
actgcatcct gacaagtgga cagtgcagcc catcgtgctg cctgagaagg acagctggac 2520
tgtgaacgac atacagaagc tcgtgggcaa gttgaactgg gcaagccaga tctacccagg 2580
catcaaagtt aggcagctgt gcaagctgct tcgaggaacc aaggcactga cagaagtgat 2640
cccactgaca gaggaagcag agctagaact ggcagagaac cgagagatcc tgaaggagcc 2700
agtacatgga gtgtactacg acccaagcaa ggacctgatc gcagagatcc agaagcaggg 2760
gcaaggccaa tggacctacc aaatctacca ggagcccttc aagaacctga agacaggcaa 2820
gtacgcaagg atgaggggtg cccacaccaa cgatgtgaag cagctgacag aggcagtgca 2880
gaagatcacc acagagagca tcgtgatctg gggcaagact cccaagttca agctgcccat 2940
acagaaggag acatgggaga catggtggac cgagtactgg caagccacct ggatccctga 3000
gtgggagttc gtgaacaccc ctcccttggt gaaactgtgg tatcagctgg agaaggaacc 3060
catcgtggga gcagagacct tctacgtgga tggggcagcc aacagggaga ccaagctggg 3120
caaggcaggc tacgtgacca accgaggacg acagaaagtg gtgaccctga ctgacaccac 3180
caaccagaag actgagctgc aagccatcta cctagctctg caagacagcg gactggaagt 3240
gaacatcgtg acagactcac agtacgcatg ggcatcatcc aagcacaacc agaccaatcc 3300
gagtcagagc tggtgaacca gatcatcgag cagctgatca agaaggagaa agtgtacctg 3360
gcatgggtac cagcacacaa aggaattgga ggaaatgaac aagtagataa attagtcagt 3420
gctgggatcc ggaaggtgct gttcctggac gggatcgata aggcccaaga tgaacatgag 3480
aagtaccact ccaactggcg cgctatggcc agcgacttca acctgccacc tgtagtagca 3540
aaagaaatag tagccagctg tgataaatgt cagctaaaag gagaagccat gcatggacaa 3600
gtagactgta gtccaggaat atggcagctg gactgcacgc acctggaggg gaaggtgatc 3660
ctggtagcag ttcatgtagc cagtggatat atagaagcag aagttatccc tgctgaaact 3720
gggcaggaaa cagcatattt tcttttaaaa ttagcaggaa gatggccagt aaaaacaata 3780
cacacggaca acggaagcaa cttcactggt gctacggtta aggccgcctg ttggtgggcg 3840
ggaatcaagc aggaatttgg aattccctac aatccccaat cgcaaggagt cgtggagagc 3900
atgaacaagg agctgaagaa gatcatcgga cagtgaggga tcaggctgag cacctgaaga 3960
cagcagtgca gatggcagtg ttcatccaca acttcaaaag aaaaggggggg attgggggggt 4020

acagtgcagg ggaaaggatc gtggacatca tcgccaccga catccaaacc aaggagctgc 4080
agaagcagat caccaagatc cagaacttcc gggtgtacta ccgcgacagc cgcaacccac 4140
tgtggaaggg accagcaaag ctcctctgga agggagaggg ggcagtggtg atccaggaca 4200
acagtgacat caaagtggtg ccaaggcgca aggccaagat catccgcgac tatggaaaac 4260
agatggcagg tgatgattgt gtggcaagta gacaggatga ggattagaac ctggaagagc 4320
ctggtgaagc accatatg 4338

<210> 2
<211> 2507
<212> DNA
<213> Human immunodeficiency virus type 1

<400> 2
tgtacagaga tggaaaagga agggaaaatt tcaaaaattg ggcctgaaaa tccatacaat 60
actccagtat ttgccataaa gaaaaaagac agtactaaat ggagaaaatt agtagatttc 120
agagaactta ataagagaac tcaagacttc tgggaagttc aattaggaat accacatccc 180
gcagggttaa aaaagaaaaa atcagtaaca gtactggatg tgggtgatgc atatttttca 240
gttcccttag atgaagactt caggaaatat actgcattta ccatacctag tataaacaat 300
gagacaccag ggattagata ccatacctag tataaacaat gagacaccag ggatttgata 360
tcagtacaat gtgcttccac agggatggaa aggatcacca gcaatattcc aaagtagcat 420
gacaaaaatc ttagagcctt ttagaaaaca aaatccagac atagttatct atcaatacat 480
ggatgatttg tatgtaggat ctgacttaga aatagggcag catagaacaa aaatagagga 540
gctgagacaa catctgttga ggtggggact taccacacca gacaaaaaac atcagaaaga 600
acctccattc ctttggatgg gttatgaact ccatcctgat aaatggacag tacagcctat 660
agtgctgcca gaaaaagaca gctggactgt caatgacata cagaagttag tggggaaatt 720
gaattgggca agtcagattt acccagggat taaagtaagg caattatgta aactccttag 780
aggaaccaaa gcactaacag aagtaatacc actaacagaa gaagcagagc tagaactggc 840
agaaaacaga gagattctaa aagaaccagt acatggagtg tattatgacc catcaaaaga 900
cttaatagca gaaatacaga agcaggggca aggccaatgg acatatcaaa tttatcaaga 960
gccatttaaa aatctgaaaa caggaaaata tgcaagaatg aggggtgccc acactaatga 1020
tgtaaaacaa ttaacagagg cagtgcaaaa aataaccaca gaaagcatag taatatgggg 1080
aaagactcct aaatttaaac tgcccataca aaaggaaaca tgggaaacat ggtggacaga 1140
gtattggcaa gccacctgga ttcctgagtg ggagtttgtt aatacccctc ctttagtgaa 1200
attatggtac cagttagaga aagaacccat agtaggagca gaaaccttct atgtagatgg 1260
ggcagctaac agggagacta aattaggaaa agcaggatat gttactaata gaggaagaca 1320
aaaagttgtc accctaactg acacaacaaa tcagaagact gagttacaag caatttatct 1380
agctttgcag gattcgggat tagaagtaaa catagtaaca gactcacaat atgcattagg 1440
aatcattcaa gcacaaccag atcaaagtga atcagagtta gtcaatcaaa taatagagca 1500
gttaataaaa aaggaaaagg tctatctggc atgggtacca gcacacaaag gaattggagg 1560
aaatgaacaa gtagataaat tagtcagtgc tggaatcagg aaagtactat ttttagatgg 1620
aatagataag gcccaagatg aacatgagaa atatcacagt aattggagag caatggctag 1680
tgattttaac ctgccacctg tagtagcaaa agaaatagta gccagctgtg ataaatgtca 1740
gctaaaagga gaagccatgc atggacaagt agactgtagt ccaggaatat ggcaactaga 1800
ttgtacacat ttagaaggaa aagttatcct ggtagcagtt catgtagcca gtggatatat 1860
agaagcagaa gttattccag cagaaacagg gcaggaaaca gcatattttc ttttaaaatt 1920
agcaggaaga tggccagtaa aaacaataca tacagacaat ggcagcaatt tcaccagtgc 1980
tacggttaag gccgcctgtt ggtgggcggg aatcaagcag gaatttggaa ttccctacaa 2040

tcccaaagt caaggagtag tagaatctat gaataaagaa ttaaagaaaa ttataggaca 2100
ggtaagagat caggctgaac atcttaagac agcagtacaa atggcagtat tcatccacaa 2160
ttttaaaaga aaaggggga ttgggggta cagtgcaggg gaaagaatag tagacataat 2220
agcaacagac atacaaacta aagaattaca aaaacaaatt acaaaaattc aaaattttcg 2280
ggtttattac agggacagca gaaatccact ttggaaagga ccagcaaagc tcctctggaa 2340
aggtgaaggg gcagtagtaa tacaagataa tagtgacata aaagtagtgc caagaagaaa 2400
agcaaagatc attagggatt atggaaaaca gatggcaggt gatgattgtg tggcaagtag 2460
acaggatgag gattagaaca tggaaaagtt tagtaaaaca ccatatg 2507

<210> 3
<211> 2467
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Mutated Human Immunodeficiency Virus - 1 Pol gene

<400> 3
tgtacagaga tggagaagga agggaagatc agcaagatcg ggcctgagaa cccctacaac 60
actccagtct tcgcaatcaa gaagaaggac agtaccaagt ggagaaagct ggtggacttc 120
agagagctga acaagagaac tcaggacttc tgggaagttc agctgggcat cccacatccc 180
gctgggttga agaagaagaa gtcagtgaca gtgctggatg tgggtgatgc ctacttctcc 240
gttcccttgg acgaggactt caggaagtac actgccttca cgatacctag catcaacaac 300
gagacaccag gcatccgcta ccagtacaac gtgctgccac agggatggaa gggatcacca 360
gccatctttc aaagcagcat gaccaagatc ctggagccct tccgcaagca aaacccagac 420
atcgtgatct atcagtacat ggacgacctc tacgtaggaa gtgacctgga gatcgggcag 480
cacaggacca agatcgagga gctgagacag catctgttga ggtggggact gaccacacca 540
gacaagaagc accagaagga acctcccttc ctgtggatgg gctacgaact gcatcctgac 600
aagtggacag tgcagcccat cgtgctgcct gagaaggaca gctggactgt gaacgacata 660
cagaagctcg tgggcaagtt gaactgggca agccagatct acccaggcat caaagttagg 720
cagctgtgca agctgcttcg aggaaccaag gcactgacag aagtgatccc actgacagag 780
gaagcagagc tagaactggc agagaaccga gagatcctga aggagccagt acatggagtg 840
tactacgacc caagcaagga cctgatcgca gagatccaga agcaggggca aggccaatgg 900
acctaccaaa tctaccagga gcccttcaag aacctgaaga caggcaagta cgcaaggatg 960
aggggtgccc acaccaacga tgtgaagcag ctgacagagg cagtgcagaa gatcaccaca 1020
gagagcatcg tgatctgggg caagactccc aagttcaagc tgcccataca gaaggagaca 1080
tgggagacat ggtggaccga gtactggcaa gccacctgga tccctgagtg ggagttcgtg 1140
aacacccctc ccttggtgaa actgtggtat cagctggaga aggaacccat cgtgggagca 1200
gagaccttct acgtggatgg ggcagccaac agggagacca agctgggcaa ggcaggctac 1260
gtgaccaacc gaggacgaca gaaagtggtg accctgactg acaccaccaa ccagaagact 1320
gagctgcaag ccatctacct agctctgcaa gacagcggac tggaagtgaa catcgtgaca 1380
gactcacagt acgcactggg catcatccaa gcacaaccag accaatccga gtcagagctg 1440
gtgaaccaga tcatcgagca gctgatcaag aaggagaaag tgtacctggc atgggtacca 1500
gcacacaaag gaattggagg aaatgaacaa gtagataaat tagtcagtgc tgggatccgg 1560
aaggtgctgt tcctggacgg gatcgataag gcccaagatg aacatgagaa gtaccactcc 1620
aactggcgcg ctatggccag cgacttcaac ctgccacctg tagtagcaaa agaaatagta 1680

gccagctgtg ataaatgtca gctaaaagga gaagccatgc atggacaagt agactgtagt 1740
ccaggaatat ggcagctgga ctgcacgcac ctggagggga aggtgatcct ggtagcagtt 1800
catgtagcca gtggatatat agaagcagaa gttatccctg ctgaaactgg gcaggaaaca 1860
gcatattttc ttttaaaatt agcaggaaga tggccagtaa aaacaataca cacggacaac 1920
ggaagcaact tcactggtgc tacggttaag gccgcctgtt ggtgggcggg aatcaagcag 1980
gaatttggaa ttccctacaa tccccaatcg caaggagtcg tggagagcat gaacaaggag 2040
ctgaagaaga tcatcggaca agtgagggat caggctgagc acctgaagac agcagtgcag 2100
atggcagtgt tcatccacaa cttcaaaaga aaaggggga ttgggggta cagtgcaggg 2160
gaaaggatcg tggacatcat cgccaccgac atccaaacca aggagctgca gaagcagatc 2220
accaagatcc agaacttccg ggtgtactac cgcgacagcc gcaacccact gtggaaggga 2280
ccagcaaagc tcctctggaa gggagagggg gcagtggtga tccaggacaa cagtgacatc 2340
aaagtggtgc caaggcgcaa ggccaagatc atccgcgact atggaaaaca gatggcaggt 2400
gatgattgtg tggcaagtag acaggatgag gattagaacc tggaagagcc tggtgaagca 2460
ccatatg 2467

<210> 4
<211> 1533
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Mutated
Simian Immunodeficiency Virus Gag gene

<400> 4
atgggcgtga gaaactccgt cttgtcaggg aagaaagcag atgaattaga aaaaattagg 60
ctacgaccca acggaaagaa aaagtacatg ttgaagcatg tagtatgggc agcaaatgaa 120
ttagatagat ttggattagc agaaagcctg ttggagaaca aagaaggatg tcaaaaaata 180
ctttcggtct tagctccatt agtgccaaca ggctcagaaa atttaaaaag cctttataat 240
actgtctgcg tcatctggtg cattcacgca gaagagaaag tgaaacacac tgaggaagca 300
aaacagatag tgcagagaca cctagtggtg gaaacaggaa ccaccgaaac catgccgaag 360
acctctcgac caacagcacc atctagcggc agaggaggaa actacccagt acagcagatc 420
ggtggtaact acgtccacct gccactgtcc ccgagaaccc tgaacgcttg ggtcaagctg 480
atcgaggaga agaagttcgg agcagaagta gtgccaggat tccaggcact gtcagaaggt 540
tgcacccccct acgacatcaa ccagatgctg aactgcgttg gagaccatca ggcggctatg 600
cagatcatcc gtgacatcat caacgaggag gctgcagatt gggacttgca gcacccacaa 660
ccagctccac aacaaggaca acttagggag ccgtcaggat cagacatcgc aggaaccacc 720
tcctcagttg acgaacagat ccagtggatg taccgtcagc agaacccgat cccagtaggc 780
aacatctacc gtcgatggat ccagctgggt ctgcagaagt gcgtccgtat gtacaacccg 840
accaacattc tagatgtaaa acaagggcca aaagagccat ttcagagcta tgtagacagg 900
ttctacaaaa gtttaagagc agaacagaca gatgcagcag taaagaattg gatgactcaa 960
acactgctga ttcaaaatgc taacccagat tgcaagctag tgctgaaggg gctgggtgtg 1020
aatcccaccc tagaagaaat gctgacggct tgtcaaggag taggggggcc gggacagaag 1080
gctagattaa tggcagaagc cctgaaagag gccctcgcac cagtgccaat ccctttgca 1140
gcagcccaac agaggggacc aagaaagcca attaagtgtt ggaattgtgg gaaagaggga 1200
cactctgcaa ggcaatgcag agccccaaga agacagggat gctggaaatg tggaaaaatg 1260
gaccatgtta tggccaaatg cccagacaga caggcgggtt ttttaggcct tggtccatgg 1320

ggaaagaagc cccgcaattt ccccatggct caagtgcatc aggggctgat gccaactgct 1380
cccccagagg acccagctgt ggatctgcta aagaactaca tgcagttggg caagcagcag 1440
agagaaaagc agagagaaag cagagagaag ccttacaagg aggtgacaga ggatttgctg 1500
cacctcaatt ctctctttgg aggagaccag tag 1533

<210> 5
<211> 1532
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Consensus sequence of mutated Simian Immunodeficiency Virus Gag gene (SIVgagDX) with wild-type SIV 239 Gag gene

<400> 5
atgggcgtga gaaactccgt cttgtcaggg aagaaagcag atgaattaga aaaaattagg 60
ctacgaccca acggaaagaa aaagtacatg ttgaagcatg tagtatgggc agcaaatgaa 120
ttagatagat ttggattagc agaaagcctg ttggagaaca aagaaggatg tcaaaaaata 180
ctttcggtct tagctccatt agtgccaaca ggctcagaaa atttaaaaag cctttataat 240
actgtctgcg tcatctggtg cattcacgca gaagagaaag tgaaacacac tgaggaagca 300
aaacagatag tgcagagaca cctagtggtg gaaacaggaa cmacmgaaac yatgccraar 360
acmwstmgac caacagcacc atctagcggc agaggaggaa aytacccagt acarcaratm 420
ggtggtaact aygtccacct gccaytrwsc ccgagaacmy traaygcytg ggtmaarytg 480
atmgaggara agaarttygg agcagaagta gtgccaggat tycaggcact gtcagaaggt 540
tgcacccccct aygacatyaa ycagatgytr aaytgygtkg gagaccatca rgcggctatg 600
cagatyatcm gwgayatyat maacgaggag gctgcagatg ggacttgcag cacccacaac 660
cagctccaca acaaggacaa cttagggagc cgtcaggatc agayatygca ggaacmacyw 720
sytcagtwga ygaacaratc cagtggatgt acmgwcarca gaacccsatm ccagtaggca 780
acatytacmg kmgatggatc carctgggky tgcaraartg ygtymgwatg tayaacccra 840
cmaacattct agatgtaaaa caagggccaa aagagccatt tcagagctat gtagacaggt 900
tctacaaaag tttaagagca gaacagacag atgcagcagt aaagaattgg atgactcaaa 960
cactgctgat tcaaaatgct aacccagatt gcaagctagt gctgaagggg ctgggtgtga 1020
atcccaccct agaagaaatg ctgacggctt gtcaaggagt agggggggccg ggacagaagg 1080
ctagattaat ggcagaagcc ctgaaagagg ccctcgcacc agtgccaatc ccttttgcag 1140
cagcccaaca gaggggacca agaaagccaa ttaagtgttg gaattgtggg aaagagggac 1200
actctgcaag gcaatgcaga gccccaagaa gacagggatg ctggaaatgt ggaaaaatgg 1260
accatgttat ggccaaatgc ccagacagac aggcgggttt tttaggcctt ggtccatggg 1320
gaaagaagcc ccgcaatttc cccatggctc aagtgcatca ggggctgatg ccaactgctc 1380
ccccagagga cccagctgtg gatctgctaa agaactacat gcagttgggc aagcagcaga 1440
gagaaaagca gagagaaagc agagagaagc cttacaagga ggtgacagag gatttgctgc 1500
acctcaattc tctctttgga ggagaccagt ag 1532

<210> 6
<211> 8366

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA sequence of the construct pCMVgagpolBNKan containing a CMV promoter, a HIV gag/pol gene and a kanamycin resistance gene

<400> 6
cctggccatt gcatacgttg tatccatatc ataatatgta catttatatt ggctcatgtc 60
caacattacc gccatgttga cattgattat tgactagtta ttaatagtaa tcaattacgg 120
ggtcattagt tcatagccca tatatggagt tccgcgttac ataacttacg gtaaatggcc 180
cgcctggctg accgcccaac gacccccgcc cattgacgtc aataatgacg tatgttccca 240
tagtaacgcc aatagggact ttccattgac gtcaatgggt ggagtattta cggtaaactg 300
cccacttggc agtacatcaa gtgtatcata tgccaagtac gccccctatt gacgtcaatg 360
acggtaaatg gcccgcctgg cattatgccc agtacatgac cttatgggac tttcctactt 420
ggcagtacat ctacgtatta gtcatcgcta ttaccatggt gatgcggttt tggcagtaca 480
tcaatgggcg tggatagcgg tttgactcac ggggatttcc aagtctccac cccattgacg 540
tcaatgggag tttgttttgg caccaaaatc aacgggactt tccaaaatgt cgtaacaact 600
ccgccccatt gacgcaaatg ggcggtaggc gtgtacggtg ggaggtctat ataagcagag 660
ctcgtttagt gaaccgtcag atcgcctgga gacgccatcc acgctgtttt gacctccata 720
gaagacaccg ggaccgatcc agcctccgcg ggcgcgcgtc gacagagaga tgggtgcgag 780
agcgtcagta ttaagcgggg gagaattaga tcgatgggaa aaaattcggt taaggccagg 840
gggaaagaag aagtacaagc taaagcacat cgtatgggca agcagggagc tagaacgatt 900
cgcagttaat cctggcctgt tagaaacatc agaaggctgt agacaaatac tgggacagct 960
acaaccatcc cttcagacag gatcagagga gcttcgatca ctatacaaca cagtagcaac 1020
cctctattgt gtgcaccagc ggatcgagat caaggacacc aaggaagctt tagacaagat 1080
agaggaagag caaaacaagt ccaagaagaa ggcccagcag gcagcagctg acacaggaca 1140
cagcaatcag gtcagccaaa attaccctat agtgcagaac atccaggggc aaatggtaca 1200
tcaggccata tcacctagaa ctttaaatgc atgggtaaaa gtagtagaag agaaggcttt 1260
cagcccagaa gtgataccca tgttttcagc attatcagaa ggagccaccc cacaggacct 1320
gaacacgatg ttgaacaccg tgggggggaca tcaagcagcc atgcaaatgt taaaagagac 1380
catcaatgag gaagctgcag aatgggatag agtgcatcca gtgcatgcag ggcctattgc 1440
accaggccag atgagagaac caaggggaag tgacatagca ggaactacta gtacccttca 1500
ggaacaaata ggatggatga caaataatcc acctatccca gtaggagaga tctacaagag 1560
gtggataatc ctgggattga acaagatcgt gaggatgtat agccctacca gcattctgga 1620
cataagacaa ggaccaaagg aacccttttag agactatgta gaccggttct ataaaactct 1680
aagagctgag caagcttcac aggaggtaaa aaattggatg acagaaacct tgttggtcca 1740
aaatgcgaac ccagattgta agaccatcct gaaggctctc ggcccagcgg ctacactaga 1800
agaaatgatg acagcatgtc agggagtagg aggacccggc cataaggcaa gagttttggc 1860
cgaggcgatg agccaggtga cgaactcggc gaccataatg atgcagagag gcaacttccg 1920
gaaccagcgg aagatcgtca agtgcttcaa ttgtggcaaa gaagggcaca ccgccaggaa 1980
ctgccgggcc ccccggaaga agggctgttg gaaatgtgga aaggaaggac accaaatgaa 2040
agattgtact gagagacagg ctaatttttt agggaagatc tggccttcct acaagggaag 2100
gccagggaat tttcttcaga gcagaccaga gccaacagcc ccaccagaag agagcttcag 2160
gtctggggta gagacaacaa ctccccctca gaagcaggag ccgatagaca aggaactgta 2220
tcctttaact tccctcagat cactctttgg caacgacccc tcgtcacagt aaggatcggg 2280

gggcaactca aggaagcgct gctcgataca ggagcagatg atacagtatt agaagaaatg 2340
agtttgccag gaagatggaa accaaaaatg atagggggga tcgggggctt catcaaggtg 2400
aggcagtacg accagatact catagaaatc tgtggacata aagctatagg tacagtatta 2460
gtaggaccta cacctgtcaa cataattgga agaaatctgt tgacccagat cggctgcacc 2520
ttgaacttcc ccatcagccc tattgagacg gtgcccgtga agttgaagcc ggggatggac 2580
ggccccaagg tcaagcaatg gccattgacg aaagagaaga tcaaggcctt agtcgaaatc 2640
tgtacagaga tggagaagga agggaagatc agcaagatcg ggcctgagaa cccctacaac 2700
actccagtct tcgcaatcaa gaagaaggac agtaccaagt ggagaaagct ggtggacttc 2760
agagagctga acaagagaac tcaggacttc tgggaagttc agctgggcat cccacatccc 2820
gctgggttga agaagaagaa gtcagtgaca gtgctggatg tgggtgatgc ctacttctcc 2880
gttcccttgg acgaggactt caggaagtac actgccttca cgatacctag catcaacaac 2940
gagacaccag gcatccgcta ccagtacaac gtgctgccac agggatggaa gggatcacca 3000
gccatctttc aaagcagcat gaccaagatc ctggagccct tccgcaagca aaacccagac 3060
atcgtgatct atcagtacat ggacgacctc tacgtaggaa gtgacctgga gatcgggcag 3120
cacaggacca agatcgagga gctgagacag catctgttga ggtggggact gaccacacca 3180
gacaagaagc accagaagga acctcccttc ctgtggatgg gctacgaact gcatcctgac 3240
aagtggacag tgcagcccat cgtgctgcct gagaaggaca gctggactgt gaacgacata 3300
cagaagctcg tgggcaagtt gaactgggca agccagatct acccaggcat caaagttagg 3360
cagctgtgca agctgcttcg aggaaccaag gcactgacag aagtgatccc actgacagag 3420
gaagcagagc tagaactggc agagaaccga gagatcctga aggagccagt acatggagtg 3480
tactacgacc caagcaagga cctgatcgca gagatccaga agcaggggca aggccaatgg 3540
acctaccaaa tctaccagga gcccttcaag aacctgaaga caggcaagta cgcaaggatg 3600
aggggtgccc acaccaacga tgtgaagcag ctgacagagg cagtgcagaa gatcaccaca 3660
gagagcatcg tgatctgggg caagactccc aagttcaagc tgcccataca gaaggagaca 3720
tgggagacat ggtggaccga gtactggcaa gccacctgga tccctgagtg ggagttcgtg 3780
aacacccctc ccttggtgaa actgtggtat cagctggaga aggaacccat cgtgggagca 3840
gagaccttct acgtggatgg ggcagccaac agggagacca agctgggcaa ggcaggctac 3900
gtgaccaacc gaggacgaca gaaagtggtg accctgactg acaccaccaa ccagaagact 3960
gagctgcaag ccatctacct agctctgcaa gacagcggac tggaagtgaa catcgtgaca 4020
gactcacagt acgcactggg catcatccaa gcacaaccag accaatccga gtcagagctg 4080
gtgaaccaga tcatcgagca gctgatcaag aaggagaaag tgtacctggc atgggtacca 4140
gcacacaaag gaattggagg aaatgaacaa gtagataaat tagtcagtgc tgggatccgg 4200
aaggtgctgt tcctggacgg gatcgataag gcccaagatg aacatgagaa gtaccactcc 4260
aactggcgcg ctatggccag cgacttcaac ctgccacctg tagtagcaaa agaaatagta 4320
gccagctgtg ataaatgtca gctaaaagga gaagccatgc atggacaagt agactgtagt 4380
ccaggaatat ggcagctgga ctgcacgcac ctggagggga aggtgatcct ggtagcagtt 4440
catgtagcca gtggatatat agaagcagaa gttatccctg ctgaaactgg gcaggaaaca 4500
gcatattttc ttttaaaatt agcaggaaga tggccagtaa aaacaataca cacggacaac 4560
ggaagcaact tcactggtgc tacggttaag gccgcctgtt ggtgggcggg aatcaagcag 4620
gaatttggaa ttccctacaa tccccaatcg caaggagtcg tggagagcat gaacaaggag 4680
ctgaagaaga tcatcggaca agtgagggat caggctgagc acctgaagac agcagtgcag 4740
atggcagtgt tcatccacaa cttcaaaaga aaaggggga ttgggggta cagtgcaggg 4800
gaaaggatcg tggacatcat cgccaccgac atccaaacca aggagctgca gaagcagatc 4860
accaagatcc agaacttccg ggtgtactac cgcgacagcc gcaacccact gtggaaggga 4920
ccagcaaagc tcctctggaa gggagagggg gcagtggtga tccaggacaa cagtgacatc 4980
aaagtggtgc caaggcgcaa ggccaagatc atccgcgact atggaaaaca gatggcaggt 5040
gatgattgtg tggcaagtag acaggatgag gattagaacc tggaagagcc tggtgaagca 5100
ccatatggcg ttcgaagcta gcctcgagat ccagatctgc tgtgccttct agttgccagc 5160

catctgttgt ttgcccctcc cccgtgcctt ccttgaccct ggaaggtgcc actcccactg 5220
tcctttccta ataaaatgag gaaattgcat cgcattgtct gagtaggtgt cattctattc 5280
tgggggggtgg ggtggggcag cacagcaagg gggaggattg ggaagacaat agcaggcatg 5340
ctggggatgc ggtgggctct atgggtaccc aggtgctgaa gaattgaccc ggttcctcct 5400
gggccagaaa gaagcaggca catccccttc tctgtgacac accctgtcca cgcccctggt 5460
tcttagttcc agccccactc ataggacact catagctcag gagggctccg ccttcaatcc 5520
cacccgctaa agtacttgga gcggtctctc cctccctcat cagcccacca aaccaaacct 5580
agcctccaag agtgggaaga aattaaagca agataggcta ttaagtgcag agggagagaa 5640
aatgcctcca acatgtgagg aagtaatgag agaaatcata gaatttcttc cgcttcctcg 5700
ctcactgact cgctgcgctc ggtcgttcgg ctgcggcgag cggtatcagc tcactcaaag 5760
gcggtaatac ggttatccac agaatcaggg gataacgcag gaaagaacat gtgagcaaaa 5820
ggccagcaaa aggccaggaa ccgtaaaaag gccgcgttgc tggcgttttt ccataggctc 5880
cgccccctg acgagcatca caaaaatcga cgctcaagtc agaggtggcg aaacccgaca 5940
ggactataaa gataccaggc gtttcccccct ggaagctccc tcgtgcgctc tcctgttccg 6000
accctgccgc ttaccggata cctgtccgcc tttctccctt cgggaagcgt ggcgctttct 6060
caatgctcac gctgtaggta tctcagttcg gtgtaggtcg ttcgctccaa gctgggctgt 6120
gtgcacgaac ccccgttca gcccgaccgc tgcgccttat ccggtaacta tcgtcttgag 6180
tccaacccgg taagacacga cttatcgcca ctggcagcag ccactggtaa caggattagc 6240
agagcgaggt atgtaggcgg tgctacagag ttcttgaagt ggtggcctaa ctacggctac 6300
actagaagga cagtatttgg tatctgcgct ctgctgaagc cagttacctt cggaaaaaga 6360
gttggtagct cttgatccgg caaacaaacc accgctggta gcggtggttt ttttgtttgc 6420
aagcagcaga ttacgcgcag aaaaaaagga tctcaagaag atcctttgat cttttctacg 6480
gggtctgacg ctcagtggaa cgaaaactca cgttaaggga ttttggtcat gagattatca 6540
aaaaggatct tcacctagat ccttttaaat taaaaatgaa gttttaaatc aatctaaagt 6600
atatatgagt aaacttggtc tgacagttac caatgcttaa tcagtgaggc acctatctca 6660
gcgatctgtc tatttcgttc atccatagtt gcctgactcc gggggggggg ggcgctgagg 6720
tctgcctcgt gaagaaggtg ttgctgactc ataccaggcc tgaatcgccc catcatccag 6780
ccagaaagtg agggagccac ggttgatgag agctttgttg taggtggacc agttggtgat 6840
tttgaacttt tgctttgcca cggaacggtc tgcgttgtcg ggaagatgcg tgatctgatc 6900
cttcaactca gcaaaagttc gatttattca acaaagccgc cgtcccgtca agtcagcgta 6960
atgctctgcc agtgttacaa ccaattaacc aattctgatt agaaaaactc atcgagcatc 7020
aaatgaaact gcaatttatt catatcagga ttatcaatac catatttttg aaaaagccgt 7080
ttctgtaatg aaggagaaaa ctcaccgagg cagttccata ggatggcaag atcctggtat 7140
cggtctgcga ttccgactcg tccaacatca atacaaccta ttaatttccc ctcgtcaaaa 7200
ataaggttat caagtgagaa atcaccatga gtgacgactg aatccggtga gaatggcaaa 7260
agcttatgca tttctttcca gacttgttca acaggccagc cattacgctc gtcatcaaaa 7320
tcactcgcat caaccaaacc gttattcatt cgtgattgcg cctgagcgag acgaaatacg 7380
cgatcgctgt taaaaggaca attacaaaca ggaatcgaat gcaaccggcg caggaacact 7440
gccagcgcat caacaatatt ttcacctgaa tcaggatatt cttctaatac ctggaatgct 7500
gttttcccgg ggatcgcagt ggtgagtaac catgcatcat caggagtacg gataaaatgc 7560
ttgatggtcg gaagaggcat aaattccgtc agccagttta gtctgaccat ctcatctgta 7620
acatcattgg caacgctacc tttgccatgt ttcagaaaca actctggcgc atcgggcttc 7680
ccatacaatc gatagattgt cgcacctgat tgcccgacat tatcgcgagc ccatttatac 7740
ccatataaat cagcatccat gttggaattt aatcgcggcc tcgagcaaga cgtttcccgt 7800
tgaatatggc tcataacacc ccttgtatta ctgtttatgt aagcagacag ttttattgtt 7860
catgatgata tatttttatc ttgtgcaatg taacatcaga gattttgaga cacaacgtgg 7920
ctttcccccc ccccccatta ttgaagcatt tatcagggtt attgtctcat gagcggatac 7980
atatttgaat gtatttagaa aaataaacaa ataggggttc cgcgcacatt tccccgaaaa 8040

gtgccacctg acgtctaaga aaccattatt atcatgacat taacctataa aaataggcgt 8100
atcacgaggc cctttcgtct cgcgcgtttc ggtgatgacg gtgaaaacct ctgacacatg 8160
cagctcccgg agacggtcac agcttgtctg taagcggatg ccgggagcag acaagcccgt 8220
cagggcgcgt cagcgggtgt tggcgggtgt cggggctggc ttaactatgc ggcatcagag 8280
cagattgtac tgagagtgca ccatatgcgg tgtgaaatac cgcacagatg cgtaaggaga 8340
aaataccgca tcagattggc tattgg 8366

<210> 7
<211> 271
<212> PRT
<213> Escherichia coli

<400> 7

```
Met Ser His Ile Gln Arg Glu Thr Ser Cys Ser Arg Pro Arg Leu Asn
  1               5                  10                  15

Ser Asn Met Asp Ala Asp Leu Tyr Gly Tyr Lys Trp Ala Arg Asp Asn
             20                  25                  30

Val Gly Gln Ser Gly Ala Thr Ile Tyr Arg Leu Tyr Gly Lys Pro Asp
         35                  40                  45

Ala Pro Glu Leu Phe Leu Lys His Gly Lys Gly Ser Val Ala Asn Asp
     50                  55                  60

Val Thr Asp Glu Met Val Arg Leu Asn Trp Leu Thr Glu Phe Met Pro
 65                  70                  75                  80

Leu Pro Thr Ile Lys His Phe Ile Arg Thr Pro Asp Asp Ala Trp Leu
                 85                  90                  95

Leu Thr Thr Ala Ile Pro Gly Lys Thr Ala Phe Gln Val Leu Glu Glu
            100                 105                 110

Tyr Pro Asp Ser Gly Glu Asn Ile Val Asp Ala Leu Ala Val Phe Leu
        115                 120                 125

Arg Arg Leu His Ser Ile Pro Val Cys Asn Cys Pro Phe Asn Ser Asp
    130                 135                 140

Arg Val Phe Arg Leu Ala Gln Ala Gln Ser Arg Met Asn Asn Gly Leu
145                 150                 155                 160

Val Asp Ala Ser Asp Phe Asp Asp Glu Arg Asn Gly Trp Pro Val Glu
                165                 170                 175

Gln Val Trp Lys Glu Met His Lys Leu Leu Pro Phe Ser Pro Asp Ser
```

```
            180                 185                 190

Val Val Thr His Gly Asp Phe Ser Leu Asp Asn Leu Ile Phe Asp Glu
        195                 200                 205

Gly Lys Leu Ile Gly Cys Ile Asp Val Gly Arg Val Gly Ile Ala Asp
    210                 215                 220

Arg Tyr Gln Asp Leu Ala Ile Leu Trp Asn Cys Leu Gly Glu Phe Ser
225                 230                 235                 240

Pro Ser Leu Gln Lys Arg Leu Phe Gln Lys Tyr Gly Ile Asp Asn Pro
                245                 250                 255

Asp Met Asn Lys Leu Gln Phe His Leu Met Leu Asp Glu Phe Phe
            260                 265                 270
```

<210> 8
<211> 8937
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA sequence
of transfer construc pmBCwCNluci

<400> 8

```
tggaagggct aatttggtcc caaaaaagac aagagatcct tgatctgtgg atctaccaca 60
cacaaggcta cttccctgat tggcagaact acacaccagg gccagggatc agatatccac 120
tgacctttgg atggtgcttc aagttagtac cagttgaacc agagcaagta gaagaggcca 180
aataaggaga gaagaacagc ttgttacacc ctatgagcca gcatgggatg gaggacccgg 240
agggagaagt attagtgtgg aagtttgaca gcctcctagc atttcgtcac atggcccgag 300
agctgcatcc ggagtactac aaagactgct gacatcgagc tttctacaag ggactttccg 360
ctggggactt tccagggagg tgtggcctgg gcgggactgg ggagtggcga gccctcagat 420
gctacatata agcagctgct ttttgcctgt actgggtctc tctggttaga ccagatctga 480
gcctgggagc tctctggcta actagggaac ccactgctta agcctcaata aagcttgcct 540
tgagtgctca aagtagtgtg tgcccgtctg ttgtgtgact ctggtaacta gagatccctc 600
agacccttt agtcagtgtg gaaaatctct agcagtggcg cccgaacagg gacttgaaag 660
cgaaagtaaa gccagaggag atctctcgac gcaggactcg gcttgctgaa gcgcgcacgg 720
caagaggcga ggggcggcgc ctgacgagga cgccaaaaat tttgactagc ggaggctaga 780
aggagagagc tcggtgcgag agcgtcagta ttaagcgggg gagaattaga tcgatgggaa 840
aaaattcggt taaggccagg gggaaagaaa aaatataaat taaaacatat agtatgggca 900
agcagggagc tagaacgatt cgcagttaat cctggcctgt tagaaacatc agaaggctgt 960
agacaaatac tgggacagct acaaccatcc cttcagacag gatcagaaga acttagatca 1020
ttatataata cagtagcaac cctctattgt gtgcatcaaa ggatagagat aaaagacacc 1080
aaggaagctt tagacaagat agaggaagag caaaacaaaa gtaagaaaaa agcacagcaa 1140
gcagcagctg acacaggaca cagcaatcag gtcagccaaa attaccctat agtgcagaac 1200
```

atccaggggc aaatggtaca tcaggccata tcacctagaa ctttaaacga taagcttggg 1260
agttccgcgt tacataactt acggtaaatg gcccgcctgg ctgaccgccc aacgaccccc 1320
gcccattgac gtcaataatg acgtatgttc ccatagtaac gccaataggg actttccatt 1380
gacgtcaatg ggtggagtat ttacggtaaa ctgcccactt ggcagtacat caagtgtatc 1440
atatgccaag tacgcccccc attgacgtca atgacggtaa atggcccgcc tggcattatg 1500
cccagtacat gaccttatgg gactttccta cttggcagta catctacgta ttagtcatcg 1560
ctattaccat ggtgatgcgg ttttggcagt acatcaatgg gcgtggatag cggtttgact 1620
cacggggatt tccaagtctc caccccattg acgtcaatgg gagtttgttt tggcaccaaa 1680
atcaacggga ctttccaaaa tgtcgtaaca actccgcccc attgacgcaa atgggcggta 1740
ggcgtgtacg gtgggaggtc tatataagca gagctcgttt agtgaaccgt cagatcgcct 1800
ggagacgcca tccacgctgt tttgacctcc atagaagaca ccgactctag aggatccatc 1860
taagtaagct tggcattccg gtactgttgg taaaatggaa gacgccaaaa acataaagaa 1920
aggcccggcg ccattctatc ctctagagga tggaaccgct ggagagcaac tgcataaggc 1980
tatgaagaga tacgccctgg ttcctggaac aattgctttt acagatgcac atatcgaggt 2040
gaacatcacg tacgcggaat acttcgaaat gtccgttcgg ttggcagaag ctatgaaacg 2100
atatgggctg aatacaaatc acagaatcgt cgtatgcagt gaaaactctc ttcaattctt 2160
tatgccggtg ttgggcgcgt tatttatcgg agttgcagtt gcgcccgcga acgacattta 2220
taatgaacgt gaattgctca acagtatgaa catttcgcag cctaccgtag tgtttgtttc 2280
caaaaagggg ttgcaaaaaa ttttgaacgt gcaaaaaaaa ttaccaataa tccagaaaat 2340
tattatcatg gattctaaaa cggattacca gggatttcag tcgatgtaca cgttcgtcac 2400
atctcatcta cctcccggtt ttaatgaata cgattttgta ccagagtcct ttgatcgtga 2460
caaaacaatt gcactgataa tgaattcctc tggatctact gggttaccta agggtgtggc 2520
ccttccgcat agaactgcct gcgtcagatt ctcgcatgcc agagatccta tttttggcaa 2580
tcaaatcatt ccggatactg cgattttaag tgttgttcca ttccatcacg gttttggaat 2640
gtttactaca ctcggatatt tgatatgtgg atttcgagtc gtcttaatgt atagatttga 2700
agaagagctg tttttacgat cccttcagga ttacaaaatt caaagtgcgt tgctagtacc 2760
aaccctattt tcattcttcg ccaaaagcac tctgattgac aaatacgatt tatctaattt 2820
acacgaaatt gcttctgggg gcgcacctct ttcgaaagaa gtcggggaag cggttgcaaa 2880
acgcttccat cttccaggga tacgacaagg atatgggctc actgagacta catcagctat 2940
tctgattaca cccgaggggg atgataaacc gggcgcggtc ggtaaagttg ttccattttt 3000
tgaagcgaag gttgtggatc tggataccgg gaaaacgctg ggcgttaatc agagaggcga 3060
attatgtgtc agaggaccta tgattatgtc cggttatgta aacaatccgg aagcgaccaa 3120
cgccttgatt gacaaggatg gatggctaca ttctggagac atagcttact gggacgaaga 3180
cgaacacttc ttcatagttg accgcttgaa gtctttaatt aaatacaaag gatatcaggt 3240
ggcccccgct gaattggaat cgatattgtt acaacacccc aacatcttcg acgcgggcgt 3300
ggcaggtctt cccgacgatg acgccggtga acttcccgcc gccgttgttg ttttggagca 3360
cggaaagacg atgacggaaa aagagatcgt ggattacgtc gccagtcaag taacaaccgc 3420
gaaaaagttg cgcggaggag ttgtgtttgt ggacgaagta ccgaaaggtc ttaccggaaa 3480
actcgacgca agaaaaatca gagagatcct cataaaggcc aagaagggcg gaaagtccaa 3540
attgtaactc gagggggggc ccggtacctt taagaccaat gacttacaag gcagctgtag 3600
atcttagcca ctttttaaaa gaaaaggggg gactggaagg gctaattcac tcccaaagaa 3660
gacaagatat ccttgatctg tggatctacc acacacaagg ctacttccct gattggcaga 3720
actacacacc agggccaggg gtcagatatc cactgacctt tggatggtgc tacaagctag 3780
taccagttga gccagataag gtagaagagg ccaataaagg agagaacacc agcttgttac 3840
accctgtgag cctgcatgga atggatgacc ctgagagaga agtgttagag tggaggtttg 3900
acagccgcct agcatttcat cacgtggccc gagagctgca tccggagtac ttcaagaact 3960
gctgacatcg agcttgctac aagggacttt ccgctgggga ctttccaggg aggcgtggcc 4020
tgggcgggac tggggagtgg cgagccctca gatgctgcat ataagcagct gctttttgcc 4080

tgtactgggt ctctctggtt agaccagatc tgagcctggg agctctctgg ctaactaggg 4140
aacccactgc ttaagcctca ataaagcttg ccttgagtgc ttcaagtagt gtgtgcccgt 4200
ctgttgtgtg actctggtaa ctagagatcc ctcagaccct tttagtcagt gtggaaaatc 4260
tctagcaccc cccaggaggt agaggttgca gtgagccaag atcgcgccac tgcattccag 4320
cctgggcaag aaaacaagac tgtctaaaat aataataata agttaagggt attaaatata 4380
tttatacatg gaggtcataa aaatatatat atttgggctg ggcgcagtgg ctcacacctg 4440
cgcccggccc tttgggaggc cgaggcaggt ggatcacctg agtttgggag ttccagacca 4500
gcctgaccaa catggagaaa ccccttctct gtgtattttt agtagatttt attttatgtg 4560
tattttattc acaggtattt ctggaaaact gaaactgttt ttcctctact ctgataccac 4620
aagaatcatc agcacagagg aagacttctg tgatcaaatg tggtgggaga gggaggtttt 4680
caccagcaca tgagcagtca gttctgccgc agactcggcg ggtgtccttc ggttcagttc 4740
caacaccgcc tgcctggaga gaggtcagac cacagggtga gggctcagtc cccaagacat 4800
aaacacccaa gacataaaca cccaacaggt ccaccccgcc tgctgcccag gcagagccga 4860
ttcaccaaga cgggaattag gatagagaaa gagtaagtca cacagagccg gctgtgcggg 4920
agaacggagt tctattatga ctcaaatcag tctccccaag cattcgggga tcagagtttt 4980
taaggataac ttagtgtgta gggggccagt gagttggaga tgaaagcgta gggagtcgaa 5040
ggtgtccttt tgcgccgagt cagttcctgg gtgggggcca caagatcgga tgagccagtt 5100
tatcaatccg ggggtgccag ctgatccatg gagtgcaggg tctgcaaaat atctcaagca 5160
ctgattgatc ttaggtttta caatagtgat gttaccccag gaacaatttg gggaaggtca 5220
gaatcttgta gcctgtagct gcatgactcc taaaccataa tttctttttt gttttttttt 5280
ttttattttt gagacagggt ctcactctgt cacctaggct ggagtgcagt ggtgcaatca 5340
cagctcactg cagcccctag agcggccgcc accgcggtgg agctccaatt cgccctatag 5400
tgagtcgtat tacaattcac tggccgtcgt tttacaacgt cgtgactggg aaaaccctgg 5460
cgttacccaa cttaatcgcc ttgcagcaca tccccctttc gccagctggc gtaatagcga 5520
agaggcccgc accgatcgcc cttcccaaca gttgcgcagc ctgaatggcg aatggcgcga 5580
aattgtaaac gttaatattt tgttaaaatt cgcgttaaat ttttgttaaa tcagctcatt 5640
ttttaaccaa taggccgaaa tcggcaaaat cccttataaa tcaaaagaat agaccgagat 5700
agggttgagt gttgttccag tttggaacaa gagtccacta ttaaagaacg tggactccaa 5760
cgtcaaaggg cgaaaaaccg tctatcaggg cgatggccca ctacgtgaac catcacccta 5820
atcaagtttt ttggggtcga ggtgccgtaa agcactaaat cggaacccta aagggagccc 5880
ccgatttaga gcttgacggg gaaagccggc gaacgtggcg agaaaggaag ggaagaaagc 5940
gaaaggagcg ggcgctaggg cgctggcaag tgtagcggtc acgctgcgcg taaccaccac 6000
acccgccgcg cttaatgcgc cgctacaggg cgcgtcccag gtggcacttt tcggggaaat 6060
gtgcgcggaa cccctatttg tttatttttc taaatacatt caaatatgta tccgctcatg 6120
agacaataac cctgataaat gcttcaataa tattgaaaaa ggaagagtat gagtattcaa 6180
catttccgtg tcgcccttat tcccttttt gcggcatttt gccttcctgt ttttgctcac 6240
ccagaaacgc tggtgaaagt aaaagatgct gaagatcagt tgggtgcacg agtgggttac 6300
atcgaactgg atctcaacag cggtaagatc cttgagagtt ttcgccccga agaacgtttt 6360
ccaatgatga gcacttttaa agttctgcta tgtggcgcgg tattatcccg tattgacgcc 6420
gggcaagagc aactcggtcg ccgcatacac tattctcaga atgacttggt tgagtactca 6480
ccagtcacag aaaagcatct tacggatggc atgacagtaa gagaattatg cagtgctgcc 6540
ataaccatga gtgataacac tgcggccaac ttacttctga caacgatcgg aggaccgaag 6600
gagctaaccg ctttttgca caacatgggg gatcatgtaa ctcgccttga tcgttgggaa 6660
ccggagctga atgaagccat accaaacgac gagcgtgaca ccacgatgcc tgtagcaatg 6720
gcaacaacgt tgcgcaaact attaactggc gaactactta ctctagcttc ccggcaacaa 6780
ttaatagact ggatggaggc ggataaagtt gcaggaccac ttctgcgctc ggcccttccg 6840
gctggctggt ttattgctga taaatctgga gccggtgagc gtgggtctcg cggtatcatt 6900
gcagcactgg ggccagatgg taagccctcc cgtatcgtag ttatctacac gacggggagt 6960

caggcaacta tggatgaacg aaatagacag atcgctgaga taggtgcctc actgattaag 7020
cattggtaac tgtcagacca agtttactca tatatacttt agattgattt aaaacttcat 7080
ttttaattta aaaggatcta ggtgaagatc ctttttgata atctcatgac caaaatccct 7140
taacgtgagt tttcgttcca ctgagcgtca gaccccgtag aaaagatcaa aggatcttct 7200
tgagatcctt ttttttctgcg cgtaatctgc tgcttgcaaa caaaaaaacc accgctacca 7260
gcggtggttt gtttgccgga tcaagagcta ccaactcttt ttccgaaggt aactggcttc 7320
agcagagcgc agataccaaa tactgtcctt ctagtgtagc cgtagttagg ccaccacttc 7380
aagaactctg tagcaccgcc tacatacctc gctctgctaa tcctgttacc agtggctgct 7440
gccagtggcg ataagtcgtg tcttaccggg ttggactcaa gacgatagtt accggataag 7500
gcgcagcggt cgggctgaac gggggttcg tgcacacagc ccagcttgga gcgaacgacc 7560
tacaccgaac tgagatacct acagcgtgag ctatgagaaa gcgccacgct tcccgaaggg 7620
agaaaggcgg acaggtatcc ggtaagcggc agggtcggaa caggagagcg cacgagggag 7680
cttccagggg gaaacgcctg gtatctttat agtcctgtcg ggtttcgcca cctctgactt 7740
gagcgtcgat ttttgtgatg ctcgtcaggg gggcggagcc tatggaaaaa cgccagcaac 7800
gcggcctttt tacggttcct ggccttttgc tggccttttg ctcacatgtt ctttcctgcg 7860
ttatcccctg attctgtgga taaccgtatt accgcctttg agtgagctga taccgctcgc 7920
cgcagccgaa cgaccgagcg cagcgagtca gtgagcgagg aagcggaaga gcgcccaata 7980
cgcaaaccgc ctctccccgc gcgttggccg attcattaat gcagctggca cgacaggttt 8040
cccgactgga aagcgggcag tgagcgcaac gcaattaatg tgagttagct cactcattag 8100
gcaccccagg ctttacactt tatgcttccg gctcgtatgt tgtgtggaat tgtgagcgga 8160
taacaatttc acacaggaaa cagctatgac catgattacg ccaagctcgg aattaaccct 8220
cactaaaggg aacaaaagct gctgcagggt ccctaactgc caagccccac agtgtgccct 8280
gaggctgccc cttccttcta gcggctgccc ccactcggct ttgctttccc tagtttcagt 8340
tacttgcgtt cagccaaggt ctgaaactag gtgcgcacag agcggtaaga ctgcgagaga 8400
aagagaccag ctttacaggg ggtttatcac agtgcaccct gacagtcgtc agcctcacag 8460
ggggtttatc acattgcacc ctgacagtcg tcagcctcac agggggttta tcacagtgca 8520
cccttacaat cattccattt gattcacaat ttttttagtc tctactgtgc ctaacttgta 8580
agttaaattt gatcagaggt gtgttcccag aggggaaaac agtatataca gggttcagta 8640
ctatcgcatt tcaggcctcc acctgggtct tggaatgtgt cccccgaggg gtgatgacta 8700
cctcagttgg atctccacag gtcacagtga cacaagataa ccaagacacc tcccaaggct 8760
accacaatgg gccgccctcc acgtgcacat ggccggagga actgccatgt cggaggtgca 8820
agcacacctg cgcatcagag tccttggtgt ggagggaggg accagcgcag cttccagcca 8880
tccacctgat gaacagaacc tagggaaagc cccagttcta cttacaccag gaaaggc 8937

<210> 9
<211> 8937
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA sequence
from transfer construct pmBCmCNluci

<400> 9
tggaagggct aatttggtcc caaaaaagac aagagatcct tgatctgtgg atctaccaca 60
cacaaggcta cttccctgat tggcagaact acacaccagg gccagggatc agatatccac 120
tgacctttgg atggtgcttc aagttagtac cagttgaacc agagcaagta gaagaggcca 180

aataaggaga gaagaacagc ttgttacacc ctatgagcca gcatgggatg gaggacccgg 240
agggagaagt attagtgtgg aagtttgaca gcctcctagc atttcgtcac atggcccgag 300
agctgcatcc ggagtactac aaagactgct gacatcgagc tttctacaag ggactttccg 360
ctggggactt tccagggagg tgtggcctgg gcgggactgg ggagtggcga gccctcagat 420
gctacatata agcagctgct ttttgcctgt actgggtctc tctggttaga ccagatctga 480
gcctgggagc tctctggcta actagggaac ccactgctta agcctcaata aagcttgcct 540
tgagtgctca aagtagtgtg tgcccgtctg ttgtgtgact ctggtaacta gagatccctc 600
agacccttt agtcagtgtg gaaaatctct agcagtggcg cccgaacagg gacttgaaag 660
cgaaagtaaa gccagaggag atctctcgac gcaggactcg gcttgctgaa gcgcgcacgg 720
caagaggcga ggggcggcgc ctgacgagga cgccaaaaat tttgactagc ggaggctaga 780
aggagagagc tcggtgcgag agcgtcagta ttaagcgggg gagaattaga tcgatgggaa 840
aaaattcggt taaggccagg gggaaagaag aagtacaagc taaagcacat cgtatgggca 900
agcagggagc tagaacgatt cgcagttaat cctggcctgt tagaaacatc agaaggctgt 960
agacaaatac tgggacagct acaaccatcc cttcagacag gatcagagga gcttcgatca 1020
ctatacaaca cagtagcaac cctctattgt gtgcaccagc ggatcgagat caaggacacc 1080
aaggaagctt tagacaagat agaggaagag caaaacaagt ccaagaagaa ggcccagcag 1140
gcagcagctg acacaggaca cagcaatcag gtcagccaaa attaccctat agtgcagaac 1200
atccaggggc aaatggtaca tcaggccata tcacctagaa ctttaaacga taagcttggg 1260
agttccgcgt tacataactt acggtaaatg gcccgcctgg ctgaccgccc aacgacccc 1320
gcccattgac gtcaataatg acgtatgttc ccatagtaac gccaataggg actttccatt 1380
gacgtcaatg ggtggagtat ttacggtaaa ctgcccactt ggcagtacat caagtgtatc 1440
atatgccaag tacgcccccc attgacgtca atgacggtaa atggcccgcc tggcattatg 1500
cccagtacat gaccttatgg gactttccta cttggcagta catctacgta ttagtcatcg 1560
ctattaccat ggtgatgcgg ttttggcagt acatcaatgg gcgtggatag cggtttgact 1620
cacggggatt tccaagtctc caccccattg acgtcaatgg gagtttgttt tggcaccaaa 1680
atcaacggga ctttccaaaa tgtcgtaaca actccgcccc attgacgcaa atgggcggta 1740
ggcgtgtacg gtgggaggtc tatataagca gagctcgttt agtgaaccgt cagatcgcct 1800
ggagacgcca tccacgctgt tttgacctcc atagaagaca ccgactctag aggatccatc 1860
taagtaagct tggcattccg gtactgttgg taaaatggaa gacgccaaaa acataaagaa 1920
aggcccggcg ccattctatc ctctagagga tggaaccgct ggagagcaac tgcataaggc 1980
tatgaagaga tacgccctgg ttcctggaac aattgctttt acagatgcac atatcgaggt 2040
gaacatcacg tacgcggaat acttcgaaat gtccgttcgg ttggcagaag ctatgaaacg 2100
atatgggctg aatacaaatc acagaatcgt cgtatgcagt gaaaactctc ttcaattctt 2160
tatgccggtg ttgggcgcgt tatttatcgg agttgcagtt gcgcccgcga acgacattta 2220
taatgaacgt gaattgctca acagtatgaa catttcgcag cctaccgtag tgtttgtttc 2280
caaaaagggg ttgcaaaaaa ttttgaacgt gcaaaaaaaa ttaccaataa tccagaaaat 2340
tattatcatg gattctaaaa cggattacca gggatttcag tcgatgtaca cgttcgtcac 2400
atctcatcta cctcccggtt ttaatgaata cgattttgta ccagagtcct ttgatcgtga 2460
caaaacaatt gcactgataa tgaattcctc tggatctact gggttaccta agggtgtggc 2520
ccttccgcat agaactgcct gcgtcagatt ctcgcatgcc agagatccta tttttggcaa 2580
tcaaatcatt ccggatactg cgattttaag tgttgttcca ttccatcacg gttttggaat 2640
gtttactaca ctcggatatt tgatatgtgg atttcgagtc gtcttaatgt atagatttga 2700
agaagagctg tttttacgat cccttcagga ttacaaaatt caaagtgcgt tgctagtacc 2760
aaccctattt tcattcttcg ccaaaagcac tctgattgac aaatacgatt tatctaattt 2820
acacgaaatt gcttctgggg gcgcacctct ttcgaaagaa gtcggggaag cggttgcaaa 2880
acgcttccat cttccaggga tacgacaagg atatgggctc actgagacta catcagctat 2940
tctgattaca cccgaggggg atgataaacc gggcgcggtc ggtaaagttg ttccattttt 3000
tgaagcgaag gttgtggatc tggataccgg gaaaacgctg ggcgttaatc agagaggcga 3060

attatgtgtc agaggaccta tgattatgtc cggttatgta aacaatccgg aagcgaccaa 3120
cgccttgatt gacaaggatg gatggctaca ttctggagac atagcttact gggacgaaga 3180
cgaacacttc ttcatagttg accgcttgaa gtctttaatt aaatacaaag gatatcaggt 3240
ggcccccgct gaattggaat cgatattgtt acaacacccc aacatcttcg acgcgggcgt 3300
ggcaggtctt cccgacgatg acgccggtga acttcccgcc gccgttgttg ttttggagca 3360
cggaaagacg atgacggaaa aagagatcgt ggattacgtc gccagtcaag taacaaccgc 3420
gaaaaagttg cgcggaggag ttgtgtttgt ggacgaagta ccgaaaggtc ttaccggaaa 3480
actcgacgca agaaaaatca gagagatcct cataaaggcc aagaagggcg gaaagtccaa 3540
attgtaactc gagggggggc ccggtacctt taagaccaat gacttacaag gcagctgtag 3600
atcttagcca ctttttaaaa gaaaaggggg gactggaagg gctaattcac tcccaaagaa 3660
gacaagatat ccttgatctg tggatctacc acacacaagg ctacttccct gattggcaga 3720
actacacacc agggccaggg gtcagatatc cactgacctt tggatggtgc tacaagctag 3780
taccagttga gccagataag gtagaagagg ccaataaagg agagaacacc agcttgttac 3840
accctgtgag cctgcatgga atggatgacc ctgagagaga agtgttagag tggaggtttg 3900
acagccgcct agcatttcat cacgtggccc gagagctgca tccggagtac ttcaagaact 3960
gctgacatcg agcttgctac aagggacttt ccgctgggga ctttccaggg aggcgtggcc 4020
tgggcgggac tggggagtgg cgagccctca gatgctgcat ataagcagct gcttttgcc 4080
tgtactgggt ctctctggtt agaccagatc tgagcctggg agctctctgg ctaactaggg 4140
aacccactgc ttaagcctca ataaagcttg ccttgagtgc ttcaagtagt gtgtgcccgt 4200
ctgttgtgtg actctggtaa ctagagatcc ctcagaccct tttagtcagt gtggaaaatc 4260
tctagcaccc cccaggaggt agaggttgca gtgagccaag atcgcgccac tgcattccag 4320
cctgggcaag aaaacaagac tgtctaaaat aataataata agttaagggt attaaatata 4380
tttatacatg gaggtcataa aaatatatat atttgggctg ggcgcagtgg ctcacacctg 4440
cgcccggccc tttgggaggc cgaggcaggt ggatcacctg agtttgggag ttccagacca 4500
gcctgaccaa catggagaaa ccccttctct gtgtattttt agtagatttt attttatgtg 4560
tattttattc acaggtattt ctggaaaact gaaactgttt ttcctctact ctgataccac 4620
aagaatcatc agcacagagg aagacttctg tgatcaaatg tggtgggaga gggaggtttt 4680
caccagcaca tgagcagtca gttctgccgc agactcggcg ggtgtccttc ggttcagttc 4740
caacaccgcc tgcctggaga gaggtcagac cacagggtga gggctcagtc cccaagacat 4800
aaacacccaa gacataaaca cccaacaggt ccaccccgcc tgctgcccag gcagagccga 4860
ttcaccaaga cgggaattag gatagagaaa gagtaagtca cacagagccg gctgtgcggg 4920
agaacggagt tctattatga ctcaaatcag tctccccaag cattcgggga tcagagtttt 4980
taaggataac ttagtgtgta gggggccagt gagttggaga tgaaagcgta gggagtcgaa 5040
ggtgtccttt tgcgccgagt cagttcctgg gtgggggcca caagatcgga tgagccagtt 5100
tatcaatccg ggggtgccag ctgatccatg gagtgcaggg tctgcaaaat atctcaagca 5160
ctgattgatc ttaggtttta caatagtgat gttaccccag gaacaatttg gggaaggtca 5220
gaatcttgta gcctgtagct gcatgactcc taaaccataa tttctttttt gtttttttt 5280
ttttattttt gagacagggt ctcactctgt cacctaggct ggagtgcagt ggtgcaatca 5340
cagctcactg cagcccctag agcggccgcc accgcggtgg agctccaatt cgccctatag 5400
tgagtcgtat tacaattcac tggccgtcgt tttacaacgt cgtgactggg aaaaccctgg 5460
cgttacccaa cttaatcgcc ttgcagcaca tccccctttc gccagctggc gtaatagcga 5520
agaggcccgc accgatcgcc cttcccaaca gttgcgcagc ctgaatggcg aatggcgcga 5580
aattgtaaac gttaatattt tgttaaaatt cgcgttaaat ttttgttaaa tcagctcatt 5640
ttttaaccaa taggccgaaa tcggcaaaat cccttataaa tcaaaagaat agaccgagat 5700
agggttgagt gttgttccag tttggaacaa gagtccacta ttaaagaacg tggactccaa 5760
cgtcaaaggg cgaaaaaccg tctatcaggg cgatggccca ctacgtgaac catcacccta 5820
atcaagtttt ttggggtcga ggtgccgtaa agcactaaat cggaacccta aagggagccc 5880
ccgatttaga gcttgacggg gaaagccggc gaacgtggcg agaaaggaag ggaagaaagc 5940

gaaaggagcg ggcgctaggg cgctggcaag tgtagcggtc acgctgcgcg taaccaccac 6000
acccgccgcg cttaatgcgc cgctacaggg cgcgtcccag gtggcacttt tcggggaaat 6060
gtgcgcggaa cccctatttg tttatttttc taaatacatt caaatatgta tccgctcatg 6120
agacaataac cctgataaat gcttcaataa tattgaaaaa ggaagagtat gagtattcaa 6180
catttccgtg tcgcccttat tcccttttt gcggcatttt gccttcctgt ttttgctcac 6240
ccagaaacgc tggtgaaagt aaaagatgct gaagatcagt tgggtgcacg agtgggttac 6300
atcgaactgg atctcaacag cggtaagatc cttgagagtt ttcgccccga agaacgtttt 6360
ccaatgatga gcacttttaa agttctgcta tgtggcgcgg tattatcccg tattgacgcc 6420
gggcaagagc aactcggtcg ccgcatacac tattctcaga atgacttggt tgagtactca 6480
ccagtcacag aaaagcatct tacggatggc atgacagtaa gagaattatg cagtgctgcc 6540
ataaccatga gtgataacac tgcggccaac ttacttctga caacgatcgg aggaccgaag 6600
gagctaaccg ctttttgca caacatgggg gatcatgtaa ctcgccttga tcgttgggaa 6660
ccggagctga atgaagccat accaaacgac gagcgtgaca ccacgatgcc tgtagcaatg 6720
gcaacaacgt tgcgcaaact attaactggc gaactactta ctctagcttc ccggcaacaa 6780
ttaatagact ggatggaggc ggataaagtt gcaggaccac ttctgcgctc ggcccttccg 6840
gctggctggt ttattgctga taaatctgga gccggtgagc gtgggtctcg cggtatcatt 6900
gcagcactgg ggccagatgg taagccctcc cgtatcgtag ttatctacac gacggggagt 6960
caggcaacta tggatgaacg aaatagacag atcgctgaga taggtgcctc actgattaag 7020
cattggtaac tgtcagacca agtttactca tatatacttt agattgattt aaaacttcat 7080
ttttaattta aaaggatcta ggtgaagatc ctttttgata atctcatgac caaaatccct 7140
taacgtgagt tttcgttcca ctgagcgtca gaccccgtag aaaagatcaa aggatcttct 7200
tgagatcctt tttttctgcg cgtaatctgc tgcttgcaaa caaaaaaacc accgctacca 7260
gcggtggttt gtttgccgga tcaagagcta ccaactcttt ttccgaaggt aactggcttc 7320
agcagagcgc agataccaaa tactgtcctt ctagtgtagc cgtagttagg ccaccacttc 7380
aagaactctg tagcaccgcc tacatacctc gctctgctaa tcctgttacc agtggctgct 7440
gccagtggcg ataagtcgtg tcttaccggg ttggactcaa gacgatagtt accggataag 7500
gcgcagcggt cgggctgaac gggggttcg tgcacacagc ccagcttgga gcgaacgacc 7560
tacaccgaac tgagatacct acagcgtgag ctatgagaaa gcgccacgct tcccgaaggg 7620
agaaaggcgg acaggtatcc ggtaagcggc agggtcggaa caggagagcg cacgagggag 7680
cttccagggg gaaacgcctg gtatctttat agtcctgtcg ggtttcgcca cctctgactt 7740
gagcgtcgat ttttgtgatg ctcgtcaggg gggcggagcc tatggaaaaa cgccagcaac 7800
gcggcctttt tacggttcct ggccttttgc tggccttttg ctcacatgtt ctttcctgcg 7860
ttatcccctg attctgtgga taaccgtatt accgcctttg agtgagctga taccgctcgc 7920
cgcagccgaa cgaccgagcg cagcgagtca gtgagcgagg aagcggaaga gcgcccaata 7980
cgcaaaccgc ctctccccgc gcgttggccg attcattaat gcagctggca cgacaggttt 8040
cccgactgga aagcgggcag tgagcgcaac gcaattaatg tgagttagct cactcattag 8100
gcaccccagg ctttacactt tatgcttccg gctcgtatgt tgtgtggaat tgtgagcgga 8160
taacaatttc acacaggaaa cagctatgac catgattacg ccaagctcgg aattaaccct 8220
cactaaaggg aacaaaagct gctgcagggt ccctaactgc caagccccac agtgtgccct 8280
gaggctgccc cttccttcta gcggctgccc ccactcggct ttgctttccc tagtttcagt 8340
tacttgcgtt cagccaaggt ctgaaactag gtgcgcacag agcggtaaga ctgcgagaga 8400
aagagaccag ctttacaggg ggtttatcac agtgcaccct gacagtcgtc agcctcacag 8460
ggggtttatc acattgcacc ctgacagtcg tcagcctcac agggggttta tcacagtgca 8520
cccttacaat cattccattt gattcacaat ttttttagtc tctactgtgc ctaacttgta 8580
agttaaattt gatcagaggt gtgttcccag aggggaaaac agtatataca gggttcagta 8640
ctatcgcatt tcaggcctcc acctgggtct tggaatgtgt cccccgaggg gtgatgacta 8700
cctcagttgg atctccacag gtcacagtga cacaagataa ccaagacacc tcccaaggct 8760
accacaatgg gccgccctcc acgtgcacat ggccggagga actgccatgt cggaggtgca 8820

agcacacctg cgcatcagag tccttggtgt ggagggaggg accagcgcag cttccagcca 8880
tccacctgat gaacagaacc tagggaaagc cccagttcta cttacaccag gaaaggc 8937

<210> 10
<211> 122
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA sequence
of the BSSHII to ClaI fragment in transfer
construct pmBCwCNluci and pmBCmCNluci

<400> 10
cgcgcacggc aagaggcgag gggcggcgcc tgacgaggac gccaaaaatt ttgactagcg 60
gaggctagaa ggagagagct cggtgcgaga gcgtcagtat taagcggggg agaattagat 120
cg 122

<210> 11
<211> 122
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA sequence
of the BSSHII to ClaI fragment in transfer
construct 3

<400> 11
cgcgcacggc aagaggcgag gggcggcgcc tggggaggac gccaaaaatt ttgactagcg 60
gaggctagaa ggagagagat gggtgcgaga gcgtcagtat taagcggggg agaattagat 120
cg 122

<210> 12
<211> 122
<212> DNA
<213> Human immunodeficiency virus type 1

<400> 12
cgcgcacggc aagaggcgag gggcggcgac tggtgagtac gccaaaaatt ttgactatcg 60
gaggctagaa ggagagagat gggtgcgaga gcgtcagtat taagcggggg agaattagat 120
cg 122

<210> 13

<211> 122
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Plurality
Consensus sequence of DNA sequence of the BSSHII
to CLaI fragment in HIV-1 and transfer constructs

<400> 13
cgcgcacggc aagaggcgag gggcggcgac tggtgagtac gccaaaaatt ttgactagcg 60
gaggctagaa ggagagagat gggtgcgaga gcgtcggtat taagcggggg agaattagat 120
aa 122

<210> 14
<211> 122
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA sequence
of construct CMVkan/R-R-SIVgp160 CTE

<400> 14
cgcgcacggc aagaggcgag gggcggcgac tggtgagtac gccaaaaatt ttgactagcg 60
gaggctagaa ggagagagat gggtgcgaga gcgtcagtat taagcggggg agaattagat 120
cg 122

<210> 15
<211> 6978
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA sequence
of construct CMVkan/R-R-SIVgp160 CTE

<400> 15
cctggccatt gcatacgttg tatccatatc ataatatgta catttatatt ggctcatgtc 60
caacattacc gccatgttga cattgattat tgactagtta ttaatagtaa tcaattacgg 120
ggtcattagt tcatagccca tatatggagt tccgcgttac ataacttacg gtaaatggcc 180
cgcctggctg accgcccaac gacccccgcc cattgacgtc aataatgacg tatgttccca 240
tagtaacgcc aatagggact ttccattgac gtcaatgggt ggagtattta cggtaaactg 300
cccacttggc agtacatcaa gtgtatcata tgccaagtac gccccctatt gacgtcaatg 360
acggtaaatg gcccgcctgg cattatgccc agtacatgac cttatgggac tttcctactt 420
ggcagtacat ctacgtatta gtcatcgcta ttaccatggt gatgcggttt tggcagtaca 480

tcaatgggcg tggatagcgg tttgactcac ggggatttcc aagtctccac cccattgacg 540
tcaatgggag tttgttttgg caccaaaatc aacgggactt tccaaaatgt cgtaacaact 600
ccgccccatt gacgcaaatg ggcggtaggc gtgtacggtg ggaggtctat ataagcagag 660
ctcgtttagt gaaccgtcag atcgcctgga gacgccatcc acgctgtttt gacctccata 720
gaagacaccg ggaccgatcc agcctccgcg ggccgcgcta agtatgggat gtcttgggaa 780
tcagctgctt atcgccatct tgcttttaag tgtctatggg atctattgta ctctatatgt 840
cacagtcttt tatggtgtac cagcttggag gaatgcgaca attcccctct tttgtgcaac 900
caagaatagg gatacttggg gaacaactca gtgcctacca gataatggtg attattcaga 960
agtggccctt aatgttacag aaagctttga tgcctggaat aatacagtca cagaacaggc 1020
aatagaggat gtatggcaac tctttgagac ctcaataaag ccttgtgtaa aattatcccc 1080
attatgcatt actatgagat gcaataaaag tgagacagat agatggggat tgacaaaatc 1140
aataacaaca acagcatcaa caacatcaac gacagcatca gcaaaagtag acatggtcaa 1200
tgagactagt tcttgtatag cccaggataa ttgcacaggc ttggaacaag agcaaatgat 1260
aagctgtaaa ttcaacatga cagggttaaa aagagacaag aaaaaagagt acaatgaaac 1320
ttggtactct gcagatttgg tatgtgaaca agggaataac actggtaatg aaagtagatg 1380
ttacatgaac cactgtaaca cttctgttat ccaagagtct tgtgacaaac attattggga 1440
tgctattaga tttaggtatt gtgcacctcc aggttatgct ttgcttagat gtaatgacac 1500
aaattattca ggctttatgc ctaaatgttc taaggtggtg gtctcttcat gcacaaggat 1560
gatggagaca cagacttcta cttggtttgg ctttaatgga actagagcag aaaatagaac 1620
ttatatttac tggcatggta gggataatag gactataatt agtttaaata agtattataa 1680
tctaacaatg aaatgtagaa gaccaggaaa taagacagtt ttaccagtca ccattatgtc 1740
tggattggtt ttccactcac aaccaatcaa tgataggcca aagcaggcat ggtgttggtt 1800
tggaggaaaa tggaaggatg caataaaaga ggtgaagcag accattgtca aacatcccag 1860
gtatactgga actaacaata ctgataaaat caatttgacg gctcctggag gaggagatcc 1920
ggaagttacc ttcatgtgga caaattgcag aggagagttc ctctactgta aaatgaattg 1980
gtttctaaat tgggtagaag ataggaatac agctaaccag aagccaaagg aacagcataa 2040
aaggaattac gtgccatgtc atattagaca aataatcaac acttggcata aagtaggcaa 2100
aaatgtttat ttgcctccaa gagagggaga cctcacgtgt aactccacag tgaccagtct 2160
catagcaaac atagattgga ttgatggaaa ccaaactaat atcaccatga gtgcagaggt 2220
ggcagaactg tatcgattgg aattgggaga ttataaatta gtagagatca ctccaattgg 2280
cttggccccc acagatgtga agaggtacac tactggtggc acctcaagaa ataaaagagg 2340
ggtctttgtg ctagggttct tgggttttct cgcaacggca ggttctgcaa tgggagccgc 2400
cagcctgacc ctcacggcac agtcccgaac tttattggct gggatagtcc aacagcagca 2460
acagctgttg gacgtggtca agagacaaca agaattgttg cgactgaccg tctggggaac 2520
aaagaacctc cagactaggg tcactgccat cgagaagtac ttaaaggacc aggcgcagct 2580
gaatgcttgg ggatgtgcgt ttagacaagt ctgccacact actgtaccat ggccaaatgc 2640
aagtctaaca ccaaagtgga acaatgagac ttggcaagag tgggagcgaa aggttgactt 2700
cttggaagaa aatataacag ccctcctaga ggaggcacaa attcaacaag agaagaacat 2760
gtatgaatta caaaagttga atagctggga tgtgtttggc aattggtttg accttgcttc 2820
ttggataaag tatatacaat atggagttta tatagttgta ggagtaatac tgttaagaat 2880
agtgatctat atagtacaaa tgctagctaa gttaaggcag gggtataggc cagtgttctc 2940
ttccccaccc tcttatttcc agcagaccca tatccaacag gacccggcac tgccaaccag 3000
agaaggcaaa gaaagagacg gtggagaagg cggtggcaac agctcctggc cttggcagat 3060
agaatatatc cactttctta ttcgtcagct tattagactc ttgacttggc tattcagtaa 3120
ctgtaggact ttgctatcga gagtatacca gatcctccaa ccaatactcc agaggctctc 3180
tgcgacccta cagaggattc gagaagtcct caggactgaa ctgacctacc tacaatatgg 3240
gtggagctat ttccatgagg cggtccaggc cgtctggaga tctgcgacag agactcttgc 3300
gggcgcgtgg ggagacttat gggagactct taggagaggt ggaagatgga tactcgcaat 3360

ccccaggagg attagacaag ggcttgagct cactctcttg tgagggacag agaattcgga 3420
tccactagtt ctagactcga ggggggggccc ggtacgagcg cttagctagc tagagaccac 3480
ctcccctgcg agctaagctg gacagccaat gacgggtaag agagtgacat ttttcactaa 3540
cctaagacag gagggccgtc agagctactg cctaatccaa agacgggtaa aagtgataaa 3600
aatgtatcac tccaacctaa gacaggcgca gcttccgagg gatttgtcgt ctgttttata 3660
tatatttaaa agggtgacct gtccggagcc gtgctgcccg gatgatgtct tggtctagac 3720
tcgaggggggg gcccggtacg atccagatct gctgtgcctt ctagttgcca gccatctgtt 3780
gtttgccccct cccccgtgcc ttccttgacc ctggaaggtg ccactcccac tgtcctttcc 3840
taataaaatg aggaaattgc atcgcattgt ctgagtaggt gtcattctat tctgggggggt 3900
ggggtggggc agcacagcaa ggggggaggat tgggaagaca atagcaggca tgctggggat 3960
gcggtgggct ctatgggtac ccaggtgctg aagaattgac ccggttcctc ctgggccaga 4020
aagaagcagg cacatcccct tctctgtgac acaccctgtc cacgcccctg gttcttagtt 4080
ccagccccac tcataggaca ctcatagctc aggagggctc cgccttcaat cccacccgct 4140
aaagtacttg gagcggtctc tccctccctc atcagcccac caaaccaaac ctagcctcca 4200
agagtgggaa gaaattaaag caagataggc tattaagtgc agagggagag aaaatgcctc 4260
caacatgtga ggaagtaatg agagaaatca tagaatttct tccgcttcct cgctcactga 4320
ctcgctgcgc tcggtcgttc ggctgcggcg agcggtatca gctcactcaa aggcggtaat 4380
acggttatcc acagaatcag gggataacgc aggaaagaac atgtgagcaa aaggccagca 4440
aaaggccagg aaccgtaaaa aggccgcgtt gctggcgttt ttccataggc tccgcccccc 4500
tgacgagcat cacaaaaatc gacgctcaag tcagaggtgg cgaaacccga caggactata 4560
aagataccag gcgtttcccc ctggaagctc cctcgtgcgc tctcctgttc cgaccctgcc 4620
gcttaccgga tacctgtccg cctttctccc ttcgggaagc gtggcgcttt ctcaatgctc 4680
acgctgtagg tatctcagtt cggtgtaggt cgttcgctcc aagctgggct gtgtgcacga 4740
acccccgtt cagcccgacc gctgcgcctt atccggtaac tatcgtcttg agtccaaccc 4800
ggtaagacac gacttatcgc cactggcagc agccactggt aacaggatta gcagagcgag 4860
gtatgtaggc ggtgctacag agttcttgaa gtggtggcct aactacggct acactagaag 4920
gacagtattt ggtatctgcg ctctgctgaa gccagttacc ttcggaaaaa gagttggtag 4980
ctcttgatcc ggcaaacaaa ccaccgctgg tagcggtggt ttttttgttt gcaagcagca 5040
gattacgcgc agaaaaaaag gatctcaaga agatcctttg atcttttcta cggggtctga 5100
cgctcagtgg aacgaaaact cacgttaagg gattttggtc atgagattat caaaaaggat 5160
cttcacctag atccttttaa attaaaaatg aagttttaaa tcaatctaaa gtatatatga 5220
gtaaacttgg tctgacagtt accaatgctt aatcagtgag gcacctatct cagcgatctg 5280
tctatttcgt tcatccatag ttgcctgact ccgggggggg ggggcgctga ggtctgcctc 5340
gtgaagaagg tgttgctgac tcataccagg cctgaatcgc cccatcatcc agccagaaag 5400
tgagggagcc acggttgatg agagctttgt tgtaggtgga ccagttggtg attttgaact 5460
tttgctttgc cacggaacgg tctgcgttgt cgggaagatg cgtgatctga tccttcaact 5520
cagcaaaagt tcgatttatt caacaaagcc gccgtcccgt caagtcagcg taatgctctg 5580
ccagtgttac aaccaattaa ccaattctga ttagaaaaac tcatcgagca tcaaatgaaa 5640
ctgcaattta ttcatatcag gattatcaat accatatttt tgaaaaagcc gtttctgtaa 5700
tgaaggagaa aactcaccga ggcagttcca taggatggca agatcctggt atcggtctgc 5760
gattccgact cgtccaacat caatacaacc tattaatttc ccctcgtcaa aaataaggtt 5820
atcaagtgag aaatcaccat gagtgacgac tgaatccggt gagaatggca aaagcttatg 5880
catttctttc cagacttgtt caacaggcca gccattacgc tcgtcatcaa aatcactcgc 5940
atcaaccaaa ccgttattca ttcgtgattg cgcctgagcg agacgaaata cgcgatcgct 6000
gttaaaagga caattacaaa caggaatcga atgcaaccgg cgcaggaaca ctgccagcgc 6060
atcaacaata ttttcacctg aatcaggata ttcttctaat acctggaatg ctgttttccc 6120
ggggatcgca gtggtgagta accatgcatc atcaggagta cggataaaat gcttgatggt 6180
cggaagaggc ataaattccg tcagccagtt tagtctgacc atctcatctg taacatcatt 6240

ggcaacgcta cctttgccat gtttcagaaa caactctggc gcatcgggct tcccatacaa 6300
tcgatagatt gtcgcacctg attgcccgac attatcgcga gcccatttat acccatataa 6360
atcagcatcc atgttggaat ttaatcgcgg cctcgagcaa gacgtttccc gttgaatatg 6420
gctcataaca ccccttgtat tactgtttat gtaagcagac agttttattg ttcatgatga 6480
tatattttta tcttgtgcaa tgtaacatca gagattttga gacacaacgt ggctttcccc 6540
ccccccccat tattgaagca tttatcaggg ttattgtctc atgagcggat acatatttga 6600
atgtatttag aaaaataaac aaataggggt tccgcgcaca tttccccgaa aagtgccacc 6660
tgacgtctaa gaaaccatta ttatcatgac attaacctat aaaaataggc gtatcacgag 6720
gccctttcgt ctcgcgcgtt tcggtgatga cggtgaaaac ctctgacaca tgcagctccc 6780
ggagacggtc acagcttgtc tgtaagcgga tgccgggagc agacaagccc gtcagggcgc 6840
gtcagcgggt gttggcgggt gtcggggctg gcttaactat gcggcatcag agcagattgt 6900
actgagagtg caccatatgc ggtgtgaaat accgcacaga tgcgtaagga gaaaataccg 6960
catcagattg gctattgg 6978

<210> 16
<211> 879
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: SIV gp160env
IN PLASMID CMVkan/R-R-SIVgp160 CTE

<400> 16

```
Met Gly Cys Leu Gly Asn Gln Leu Leu Ile Ala Ile Leu Leu Leu Ser
  1               5                  10                  15

Val Tyr Gly Ile Tyr Cys Thr Leu Tyr Val Thr Val Phe Tyr Gly Val
             20                  25                  30

Pro Ala Trp Arg Asn Ala Thr Ile Pro Leu Phe Cys Ala Thr Lys Asn
         35                  40                  45

Arg Asp Thr Trp Gly Thr Thr Gln Cys Leu Pro Asp Asn Gly Asp Tyr
     50                  55                  60

Ser Glu Val Ala Leu Asn Val Thr Glu Ser Phe Asp Ala Trp Asn Asn
 65                  70                  75                  80

Thr Val Thr Glu Gln Ala Ile Glu Asp Val Trp Gln Leu Phe Glu Thr
                 85                  90                  95

Ser Ile Lys Pro Cys Val Lys Leu Ser Pro Leu Cys Ile Thr Met Arg
            100                 105                 110

Cys Asn Lys Ser Glu Thr Asp Arg Trp Gly Leu Thr Lys Ser Ile Thr
        115                 120                 125
```

```
Thr Thr Ala Ser Thr Thr Ser Thr Thr Ala Ser Ala Lys Val Asp Met
    130                 135                 140

Val Asn Glu Thr Ser Ser Cys Ile Ala Gln Asp Asn Cys Thr Gly Leu
145                 150                 155                 160

Glu Gln Glu Gln Met Ile Ser Cys Lys Phe Asn Met Thr Gly Leu Lys
                165                 170                 175

Arg Asp Lys Lys Lys Glu Tyr Asn Glu Thr Trp Tyr Ser Ala Asp Leu
            180                 185                 190

Val Cys Glu Gln Gly Asn Asn Thr Gly Asn Glu Ser Arg Cys Tyr Met
        195                 200                 205

Asn His Cys Asn Thr Ser Val Ile Gln Glu Ser Cys Asp Lys His Tyr
    210                 215                 220

Trp Asp Ala Ile Arg Phe Arg Tyr Cys Ala Pro Pro Gly Tyr Ala Leu
225                 230                 235                 240

Leu Arg Cys Asn Asp Thr Asn Tyr Ser Gly Phe Met Pro Lys Cys Ser
                245                 250                 255

Lys Val Val Val Ser Ser Cys Thr Arg Met Met Glu Thr Gln Thr Ser
            260                 265                 270

Thr Trp Phe Gly Phe Asn Gly Thr Arg Ala Glu Asn Arg Thr Tyr Ile
        275                 280                 285

Tyr Trp His Gly Arg Asp Asn Arg Thr Ile Ile Ser Leu Asn Lys Tyr
    290                 295                 300

Tyr Asn Leu Thr Met Lys Cys Arg Arg Pro Gly Asn Lys Thr Val Leu
305                 310                 315                 320

Pro Val Thr Ile Met Ser Gly Leu Val Phe His Ser Gln Pro Ile Asn
                325                 330                 335

Asp Arg Pro Lys Gln Ala Trp Cys Trp Phe Gly Gly Lys Trp Lys Asp
            340                 345                 350

Ala Ile Lys Glu Val Lys Gln Thr Ile Val Lys His Pro Arg Tyr Thr
        355                 360                 365

Gly Thr Asn Asn Thr Asp Lys Ile Asn Leu Thr Ala Pro Gly Gly Gly
    370                 375                 380
```

```
Asp Pro Glu Val Thr Phe Met Trp Thr Asn Cys Arg Gly Glu Phe Leu
385                 390                 395                 400

Tyr Cys Lys Met Asn Trp Phe Leu Asn Trp Val Glu Asp Arg Asn Thr
                405                 410                 415

Ala Asn Gln Lys Pro Lys Glu Gln His Lys Arg Asn Tyr Val Pro Cys
            420                 425                 430

His Ile Arg Gln Ile Ile Asn Thr Trp His Lys Val Gly Lys Asn Val
        435                 440                 445

Tyr Leu Pro Pro Arg Glu Gly Asp Leu Thr Cys Asn Ser Thr Val Thr
    450                 455                 460

Ser Leu Ile Ala Asn Ile Asp Trp Ile Asp Gly Asn Gln Thr Asn Ile
465                 470                 475                 480

Thr Met Ser Ala Glu Val Ala Glu Leu Tyr Arg Leu Glu Leu Gly Asp
                485                 490                 495

Tyr Lys Leu Val Glu Ile Thr Pro Ile Gly Leu Ala Pro Thr Asp Val
            500                 505                 510

Lys Arg Tyr Thr Thr Gly Gly Thr Ser Arg Asn Lys Arg Gly Val Phe
        515                 520                 525

Val Leu Gly Phe Leu Gly Phe Leu Ala Thr Ala Gly Ser Ala Met Gly
    530                 535                 540

Ala Ala Ser Leu Thr Leu Thr Ala Gln Ser Arg Thr Leu Leu Ala Gly
545                 550                 555                 560

Ile Val Gln Gln Gln Gln Gln Leu Leu Asp Val Val Lys Arg Gln Gln
                565                 570                 575

Glu Leu Leu Arg Leu Thr Val Trp Gly Thr Lys Asn Leu Gln Thr Arg
            580                 585                 590

Val Thr Ala Ile Glu Lys Tyr Leu Lys Asp Gln Ala Gln Leu Asn Ala
        595                 600                 605

Trp Gly Cys Ala Phe Arg Gln Val Cys His Thr Thr Val Pro Trp Pro
    610                 615                 620

Asn Ala Ser Leu Thr Pro Lys Trp Asn Asn Glu Thr Trp Gln Glu Trp
625                 630                 635                 640
```

Glu Arg Lys Val Asp Phe Leu Glu Glu Asn Ile Thr Ala Leu Leu Glu
645 650 655

Glu Ala Gln Ile Gln Gln Glu Lys Asn Met Tyr Glu Leu Gln Lys Leu
660 665 670

Asn Ser Trp Asp Val Phe Gly Asn Trp Phe Asp Leu Ala Ser Trp Ile
675 680 685

Lys Tyr Ile Gln Tyr Gly Val Tyr Ile Val Val Gly Val Ile Leu Leu
690 695 700

Arg Ile Val Ile Tyr Ile Val Gln Met Leu Ala Lys Leu Arg Gln Gly
705 710 715 720

Tyr Arg Pro Val Phe Ser Ser Pro Pro Ser Tyr Phe Gln Gln Thr His
725 730 735

Ile Gln Gln Asp Pro Ala Leu Pro Thr Arg Glu Gly Lys Glu Arg Asp
740 745 750

Gly Gly Glu Gly Gly Gly Asn Ser Ser Trp Pro Trp Gln Ile Glu Tyr
755 760 765

Ile His Phe Leu Ile Arg Gln Leu Ile Arg Leu Leu Thr Trp Leu Phe
770 775 780

Ser Asn Cys Arg Thr Leu Leu Ser Arg Val Tyr Gln Ile Leu Gln Pro
785 790 795 800

Ile Leu Gln Arg Leu Ser Ala Thr Leu Gln Arg Ile Arg Glu Val Leu
805 810 815

Arg Thr Glu Leu Thr Tyr Leu Gln Tyr Gly Trp Ser Tyr Phe His Glu
820 825 830

Ala Val Gln Ala Val Trp Arg Ser Ala Thr Glu Thr Leu Ala Gly Ala
835 840 845

Trp Gly Asp Leu Trp Glu Thr Leu Arg Arg Gly Gly Arg Trp Ile Leu
850 855 860

Ala Ile Pro Arg Arg Ile Arg Gln Gly Leu Glu Leu Thr Leu Leu
865 870 875

<210> 17

<211> 271
<212> PRT
<213> Escherichia coli

<400> 17

```
Met Ser His Ile Gln Arg Glu Thr Ser Cys Ser Arg Pro Arg Leu Asn
  1               5                  10                  15

Ser Asn Met Asp Ala Asp Leu Tyr Gly Tyr Lys Trp Ala Arg Asp Asn
             20                  25                  30

Val Gly Gln Ser Gly Ala Thr Ile Tyr Arg Leu Tyr Gly Lys Pro Asp
         35                  40                  45

Ala Pro Glu Leu Phe Leu Lys His Gly Lys Gly Ser Val Ala Asn Asp
     50                  55                  60

Val Thr Asp Glu Met Val Arg Leu Asn Trp Leu Thr Glu Phe Met Pro
 65                  70                  75                  80

Leu Pro Thr Ile Lys His Phe Ile Arg Thr Pro Asp Asp Ala Trp Leu
                 85                  90                  95

Leu Thr Thr Ala Ile Pro Gly Lys Thr Ala Phe Gln Val Leu Glu Glu
            100                 105                 110

Tyr Pro Asp Ser Gly Glu Asn Ile Val Asp Ala Leu Ala Val Phe Leu
        115                 120                 125

Arg Arg Leu His Ser Ile Pro Val Cys Asn Cys Pro Phe Asn Ser Asp
    130                 135                 140

Arg Val Phe Arg Leu Ala Gln Ala Gln Ser Arg Met Asn Asn Gly Leu
145                 150                 155                 160

Val Asp Ala Ser Asp Phe Asp Asp Glu Arg Asn Gly Trp Pro Val Glu
                165                 170                 175

Gln Val Trp Lys Glu Met His Lys Leu Leu Pro Phe Ser Pro Asp Ser
            180                 185                 190

Val Val Thr His Gly Asp Phe Ser Leu Asp Asn Leu Ile Phe Asp Glu
        195                 200                 205

Gly Lys Leu Ile Gly Cys Ile Asp Val Gly Arg Val Gly Ile Ala Asp
    210                 215                 220

Arg Tyr Gln Asp Leu Ala Ile Leu Trp Asn Cys Leu Gly Glu Phe Ser
```

225 230 235 240

Pro Ser Leu Gln Lys Arg Leu Phe Gln Lys Tyr Gly Ile Asp Asn Pro
245 250 255

Asp Met Asn Lys Leu Gln Phe His Leu Met Leu Asp Glu Phe Phe
260 265 270

<210> 18
<211> 2640
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA sequence of mutated SIV gene in construct CMVkan/R-R-SIVgp160 CTE

<400> 18
atgggatgtc ttgggaatca gctgcttatc gccatcttgc ttttaagtgt ctatgggatc 60
tattgtactc tatatgtcac agtcttttat ggtgtaccag cttggaggaa tgcgacaatt 120
cccctctttt gtgcaaccaa gaatagggat acttggggaa caactcagtg cctaccagat 180
aatggtgatt attcagaagt ggcccttaat gttacagaaa gctttgatgc ctggaataat 240
acagtcacag aacaggcaat agaggatgta tggcaactct ttgagacctc aataaagcct 300
tgtgtaaaat tatccccatt atgcattact atgagatgca ataaaagtga gacagataga 360
tggggattga caaaatcaat aacaacaaca gcatcaacaa catcaacgac agcatcagca 420
aaagtagaca tggtcaatga gactagttct tgtatagccc aggataattg cacaggcttg 480
gaacaagagc aaatgataag ctgtaaattc aacatgacag ggttaaaaag agacaagaaa 540
aaagagtaca atgaaacttg gtactctgca gatttggtat gtgaacaagg gaataacact 600
ggtaatgaaa gtagatgtta catgaaccac tgtaacactt ctgttatcca agagtcttgt 660
gacaaacatt attgggatgc tattagattt aggtattgtg cacctccagg ttatgctttg 720
cttagatgta atgacacaaa ttattcaggc tttatgccta aatgttctaa ggtggtggtc 780
tcttcatgca caaggatgat ggagacacag acttctactt ggtttggctt taatggaact 840
agagcagaaa atagaactta tatttactgg catggtaggg ataataggac tataattagt 900
ttaaataagt attataatct aacaatgaaa tgtagaagac caggaaataa gacagtttta 960
ccagtcacca ttatgtctgg attggttttc cactcacaac caatcaatga taggccaaag 1020
caggcatggt gttggtttgg aggaaaatgg aaggatgcaa taaaagaggt gaagcagacc 1080
attgtcaaac atcccaggta tactggaact aacaatactg ataaaatcaa tttgacggct 1140
cctggaggag gagatccgga agttaccttc atgtggacaa attgcagagg agagttcctc 1200
tactgtaaaa tgaattggtt tctaaattgg gtagaagata ggaatacagc taaccagaag 1260
ccaaaggaac agcataaaag gaattacgtg ccatgtcata ttagacaaat aatcaacact 1320
tggcataaag taggcaaaaa tgtttatttg cctccaagag agggagacct cacgtgtaac 1380
tccacagtga ccagtctcat agcaaacata gattggattg atggaaacca aactaatatc 1440
accatgagtg cagaggtggc agaactgtat cgattggaat tgggagatta taaattagta 1500
gagatcactc caattggctt ggcccccaca gatgtgaaga ggtacactac tggtggcacc 1560
tcaagaaata aaagaggggt ctttgtgcta gggttcttgg gttttctcgc aacggcaggt 1620
tctgcaatgg gagccgccag cctgaccctc acggcacagt cccgaacttt attggctggg 1680

atagtccaac agcagcaaca gctgttggac gtggtcaaga gacaacaaga attgttgcga 1740
ctgaccgtct ggggaacaaa gaacctccag actagggtca ctgccatcga gaagtactta 1800
aaggaccagg cgcagctgaa tgcttgggga tgtgcgttta gacaagtctg ccacactact 1860
gtaccatggc caaatgcaag tctaacacca aagtggaaca atgagacttg gcaagagtgg 1920
gagcgaaagg ttgacttctt ggaagaaaat ataacagccc tcctagagga ggcacaaatt 1980
caacaagaga agaacatgta tgaattacaa aagttgaata gctgggatgt gtttggcaat 2040
tggtttgacc ttgcttcttg gataaagtat atacaatatg gagtttatat agttgtagga 2100
gtaatactgt taagaatagt gatctatata gtacaaatgc tagctaagtt aaggcagggg 2160
tataggccag tgttctcttc cccaccctct tatttccagc agacccatat ccaacaggac 2220
ccggcactgc caaccagaga aggcaaagaa agagacggtg gagaaggcgg tggcaacagc 2280
tcctggcctt ggcagataga atatatccac tttcttattc gtcagcttat tagactcttg 2340
acttggctat tcagtaactg taggactttg ctatcgagag tataccagat cctccaacca 2400
atactccaga ggctctctgc gaccctacag aggattcgag aagtcctcag gactgaactg 2460
acctacctac aatatgggtg gagctatttc catgaggcgg tccaggccgt ctggagatct 2520
gcgacagaga ctcttgcggg cgcgtgggga gacttatggg agactcttag gagaggtgga 2580
agatggatac tcgcaatccc caggaggatt agacaagggc ttgagctcac tctcttgtga 2640

<210> 19
<211> 813
<212> DNA
<213> Escherichia coli

<400> 19
atgagccata ttcaacggga aacgtcttgc tcgaggccgc gattaaattc caacatggat 60
gctgatttat atgggtataa atgggctcgc gataatgtcg ggcaatcagg tgcgacaatc 120
tatcgattgt atgggaagcc cgatgcgcca gagttgtttc tgaaacatgg caaaggtagc 180
gttgccaatg atgttacaga tgagatggtc agactaaact ggctgacgga atttatgcct 240
cttccgacca tcaagcattt tatccgtact cctgatgatg catggttact caccactgcg 300
atccccggga aaacagcatt ccaggtatta gaagaatatc ctgattcagg tgaaaatatt 360
gttgatgcgc tggcagtgtt cctgcgccgg ttgcattcga ttcctgtttg taattgtcct 420
tttaacagcg atcgcgtatt tcgtctcgct caggcgcaat cacgaatgaa taacggtttg 480
gttgatgcga gtgattttga tgacgagcgt aatggctggc ctgttgaaca agtctggaaa 540
gaaatgcata agcttttgcc attctcaccg gattcagtcg tcactcatgg tgatttctca 600
cttgataacc ttatttttga cgaggggaaa ttaataggtt gtattgatgt tggacgagtc 660
ggaatcgcag accgatacca ggatcttgcc atcctatgga actgcctcgg tgagttttct 720
ccttcattac agaaacggct ttttcaaaaa tatggtattg ataatcctga tatgaataaa 780
ttgcagtttc atttgatgct cgatgagttt ttc 813

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/17258

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12N15/00, 63; A61K 48/00
US CL :435/325, 320.1; 514/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/325, 320.1; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98/46083 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 22 OCTOBER 1998, page 8 through page 9.	1-39NO
Y	WO 93/20212 A1 (THE GOVERNMENT OF THE UNITED STATES OF AMERICA AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES) 14 OCTOBER 1993, entire disclosure, specially abstract.	1-39
Y	NGO et al. Comutational Complexity, Protein Structure Prediction, and the Levinthal Paradox. THE PROTEIN FOLDING PROBLEM AND TERTIARY STRUCTURE PREDICTION. K. Merz, Jr. and S. Le Grand, Editors. 1994, pages 491-495, especially pages 492 and 495.	1-39

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 02 AUGUST 2002	Date of mailing of the international search report 09 SEP 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVE NGUYEN Telephone No. (703) 308-9106

Form PCT/ISA/210 (second sheet) (July 1998)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/17258

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	VERMA et al. Gene Therapy - promises, problems and prospects. Nature. 18 September 1997. Vol. 389, pages 239-242, particularly page 239-241.	1-39
A	ANDERSON. Human Gene Therapy. Nature. 30 April 1998. Vol. 392, Supp, pages 25-30, especially pages 25-26, and 30.	1-39